dialrel

Report on good and adverse practices
- Animal welfare concerns
in relation to slaughter practices
from the viewpoint of veterinary sciences

Report

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<u>Table of Contents:</u>

1   Introduction

This report as part of the dialogue on religious slaughter summarises the animal welfare concerns from the viewpoint of veterinary sciences in relation to slaughter practices.
It includes *neck cutting without stunning*, *stunning prior to neck cutting* (in the context of religious slaughter), and *post neck cut stunning* .

The aim is to discuss and evaluate the different types of slaughter practices, including pre-slaughter handling. This report has been produced in an unbiased and comparative manner, taking into account scientific findings and observations gathered by veterinarians and scientists under practical conditions.

Part of the report will also be based on observations made during the spot visits, carried out during the project in Germany, Spain, Great Britain, France, Belgium, Italy, Netherlands, Israel, Australia (and New Zealand). This is referred to as experience gathered by the veterinarians of the Dialrel consortium, mainly during WP2[1]. Species covered are cattle, sheep, goats and poultry (predominately chicken and turkey).

2   Physiological basics

2.1 Pain

Broom (2001) described pain as an aversive sensation and feeling associated with actual or potential tissue damage. This description was developed from the definition of the International Association for the study of pain (IASP) which states that "Pain is an unpleasant sensory or emotional experience associated with actual or potential tissue damage, or described in term of such damage" (IASP, 1979). "Aversive" is used instead of "unpleasant" because aversion is more readily recognised and assessed than unpleasantness, particularly in non-human species and "feeling" implies some degree of awareness unlike "emotion" (Broom, 2001).

The neuropsychological system that regulates the perception of pain in man and animals (nociceptive system) has been suggested as an evolutionary protective system. It has adaptive value in escape and avoidance or during repair and recuperation. The function of the nociceptive system is similar in all mammalian species and also birds. Differences between man and animals can be found in the cognitive operated reactions to end, to avoid and to cope with a condition of pain (Zimmermann, 2005; Broom, 2001). But also an emotional component of pain is suggested for mammalians as well as poultry (Serviere et al., 2009)[2]. Though emotional awareness is not necessarily required for nociceptive responses it can be assumed that vertebrates are conscious of pain (Walters, 2008).

Nociception is the general process of encoding and processing of <u>noxious stimuli</u> by the central nervous system. A noxious stimulus is an actually or potentially tissue damaging event. Tissue damage can be caused by a variety of stimuli, including physical, mechanical, chemical and temperature. Although tissue damage is the common denominator of those stimuli that may cause pain, there are some types of tissue damage that do not stimulate nociceptors, and thus do not activate the nociceptive system and cause pain. Furthermore some tissues are devoid of nociceptors (e.g. brain). In some situations tissue damage can

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[1] WP2 is workpackage 2 of Dialrel "Assessment of current practices", monitors the current state and examines and discusses the evidence from observed (spot visits) or reported (questionnaires) incidences of optimum and adverse practices of religious slaughter techniques

[2] A comprehensive interdisciplinary report on identification, understanding and limiting of pain in farmed livestock was recently produced by Le Neidre et al. (2009).

occur, but the damage is not perceived as pain, as the tissue damage does not activate nociceptors, and thus does not cause pain or any protective behavioural changes. This is a well-known phenomenon in internal organs such as the liver or the brain, where a malignant tumour may cause extensive damage that goes unnoticed by the patient (Treede, 2008). The meninges of the brain are nevertheless sensitive and a brain swelling caused by the tumour may cause pain due to activation of nerve endings associated with the meninges.

The perception of pain is based on an interaction of receptors, nerves, the spinal cord and the brain including the thalamus and the cortex (Brooks and Tracey, 2005; Treede et al., 2000). Pain receptors are located in skin, muscles, joints, periosteum, most internal organs and around blood vessels. Pain can lead to different experiences (e.g. sharp, dull) as different anatomical structures are involved, and different tissues are characterized by different sensors, density of sensors and different types of fibres. Sharp pain is signaled by A-fibers (conduction time 5-30 m/s) and the reaction time for perception of sharp pain is short. C-fibers (conduction time 0,5-2m/s) are associated with a slower burning type of pain. Both types of nociceptive fibres innervate the skin and deep somatic or visceral structures (Ringkamp and Meyer, 2008; Hellyer et al., 2007).

During the slaughter process itself pain can be caused by inappropriate restraint, during incorrectly performed stuns and by tissue damage during the neck cut. There are different types of pain, of which two are welfare relevant during the short time frame of the slaughter process. Phasic or nociceptive pain results from mechanical or thermal stimuli is also called "brief" or "first pain". Tonic or inflammatory type of pain resulting from chemical stimuli released by injury and inflammations is also called "persistent" or "second pain". During slaughter both forms of pain are produced. Nociceptive pain is produced by mechanical forces of cutting and inflammatory pain immediately thereafter by tissue damage. The severity of inflammatory pain can be reduced but not eliminated by a clean cut performed with a sharp knife, while this has little or no influence on nociceptive pain (Brooks and Tracey, 2005; Woolf, 2004).

The threshold of nociceptors is not constant. Substances from damaged cells or inflamed tissues directly stimulate nociceptors and are considered "nociceptive activators" (e.g. potassium ions or ATP or certain inflammatory mediators). These substances contribute to primary hyperalgesia. A so called "sensitizing soup" sensitizes the nociceptors to subsequent painful and also nonpainful stimuli (Muir, 2007; Hellyer et al., 2007).

Pain can be modulated by the central nervous system in both directions (Tracey and Mantyh, 2007). Not all traumata are directly painful, as stress can inhibit the transmission of pain stimuli in brain and spinal cord (Gregory, 2004). This phenomenon called stress-induced analgesia is part of the bodies self protection measures during life-threatening situations, it involves endogenous opioides, which block pain neurotransmission (Zimmermann, 2005). It must be considered in this context that stress induced analgesia does not apply in every life threatening situation and for every individual. Often this involves the individual being involved in very vigorous activity and heightened awareness, frequently associated with emergency physiological responses. This can apply to fighting or other dangerous and demanding activities (Bodnar, 1984). The possibility exists that animals which are to be slaughtered might be in such a state but with correct pre-slaughter handling this would not be the routine situation. Furthermore, only around 30-40% of humans experience stress induced analgesia in an emergency situation (Melzack et al., 1982). Hence it is likely that endogenous-opioid-induced analgesia may not often occur during slaughter. This can be underlined by practitioners reports of animal pain reactions during stressful situations. Cattle for example, being restrained for claw trimming and showing obvious stress symptoms (wide open eyes, vocalisation) still react immensely when e.g. the bandage is taken off an inflamed claw.

On the other hand nociceptive stimulation of medullary brain centers produces reflex responses including hyperventilation, increased sympathetic tone and catecholamine similar to the stress response, which are further increased by anxiety and fear. Thus attenuation of the stress response is recommended in veterinary anesthesia (Hellyer et al., 2007).

2.1.1 Expression of pain

Animals can express pain in the following ways (Gregory, 2004):
- Escape reactions
- Immobility
- Abnormal posture, gait or speed, guarding behaviour
- Vocalising or aggression during movement or manipulation
- Withdrawal and recoil responses
- Licking, biting, chewing or scratching
- Frequent changes in body position – restlessness, rolling, writhing, kicking, tail-flicking
- Vocalising – groaning, whimpering, crying, squealing, screaming, growling, hissing, barking
- Impaired breathing pattern, shallow breathing, groaning during breathing, increased rate of breathing
- Muscle tension, tremor, twitching, spasm, straining
- Depression, sluggishness, hiding, withdrawal, lying motionless, seeking cover, sleeplessness
- Avoidance behaviour and aversion to the scene of the trauma
- Spontaneous autonomic responses – sweating, tachycardia, bradycardia hypertension, vasoconstriction and pallor, increased gastro-intestinal secretions, decreased intestinal motility, increased intestinal sphincter tone, urinary retention
- Endocrine responses (se below).

The expression of pain differs not only from species to species, but also from individual to individual. Prey species, which live in flocks (e.g. sheep), normally only show very faint signs of pain, as obviously weak or injured animals might attract predators. Individuals within a species vary in the thresholds for the elicitation of pain responses (Gregory, 2004; Broom, 2001).

Recognizing pain can be difficult, because different pain levels or qualities may be expressed differently (Grant, 2004) and some of the signs are not only motivated by pain, like tail wagging and vocalisation (Gregory, 2005b; Grant, 2004; Molony et al., 1995; Molony et al., 1993). During slaughter pain reactions may be masked by restraining device or when the animal is shackled (Holleben, 2009), also the animal may not be able to express a normal response to pain because of the process of slaughter (animals are unable to vocalize if their throat is cut).

2.1.2 Physiological indices for pain

Additionally to the aforementioned way, pain can be expressed by animals through their clinical appearance and behaviour. The following list of physiological indices for pain are mentioned by Mellor et al. (2000):
- blood hormone concentrations like adrenaline, noradrenaline, corticotropin releasing factor, adrenocorticotropic hormone, glucocorticoids (e.g. cortisol), prolactin concentrations
- Blood metabolite concentrations like glucose, lactic acid, free fatty acids, β-hydroxybutyrate

- Other variables: heart rate, breathing (rate and depth), packed cell volume, sweat production, muscle tremor, body temperature, plasma α-acid glycoprotein levels, blood leukocyte levels, cellular immune responses, humoral immune responses

Most of these parameters are not suitable for the study of pain directly following the neck cut during slaughter. This is because they are not specific to pain (e.g. heart rate, hormone responses), the time course is too short for a meaningful response to be expressed (e.g. hormone responses), or features in the process of slaughter inherently confound the measure (e.g. heart rate, blood pressure), or prevent expression of the measure (e.g. vocalisation) (Hemsworth et al., 2009).

A recent review by Gregory (2010) brought together cases where quantitative relationships between pain and pathology severities have been established in human medicine. The findings on ulcers, cysts and organomegaly imply that there is a quantitative relationship which either involves a threshold at which pain is evoked by tissue stretching or a gradation in pain severity with lesion size.

2.2 Fear

Fear is an unpleasant emotional condition when anticipating a highly negative event (Sambraus, 1997). Fear and anxiety are two emotional states induced by perception of danger or potential danger, respectively, that threaten the integrity of the animal (Jones et al., 2000; Boissy, 1995). Fear and anxiety both involve physiological and behavioural changes that prepare the animal to cope with the danger. Although fear and anxiety have not always been clearly differentiated, fear can be operationally defined as a state of apprehension focussing on isolated and recognisable dangers while anxieties are diffuse states of tension that magnify the illusion of unseen dangers (Rowan, 1988). General fear becomes a problem particularly when animals encounter new or unexpected stimuli (e.g. a sudden noise or movement, an unfamiliar animal), or situations, e.g. during handling or transportation. This has important implications for animal housing and management. For example, inappropriate handling, corridors/races and pen design, discontinuities in floor texture and colour, drafts and (poor) lighting may all induce fear and its undesirable consequences (Grandin, 2000).

There are four types of fear commonly recognised in animals:
- Innate fears – e.g. isolation, fear of the dark, snakes, spiders;
- Novelty – e.g. strange objects, sudden movements;
- Fears learned by experience – anticipated pain;
- Fear provoked by signs of fear in others;

Things which are very frightening for one species may be only mildly so for another. Fear may result in panic attacks, which in humans are defined as a sudden fear accompanied by a feeling of terror and an intense urge to escape. In flock animals collective panic resulting in wild flight impossible to stop can be started by a single animal sometimes provoked by trivial causes like insects (Gregory, 2004).

Fear, anxiety and excitement can heighten the experience of pain via activation the sympathetic autonomous nervous system (Tracey and Mantyh, 2007). Fear and excitement are also important for the effectiveness of stunning methods as they may have an impact on correct positioning of devices and the effectiveness of exsanguination (see below).

2.2.1 Expression of fear

The expression of fear differs widely from species to species and according to individual and genetic differences (Grignard et al., 2001; Boissy and Bouissou, 1995; Boivin et al., 1994; Grandin, 1993a). Fear in animals can be shown by wide open eyes, freezing reactions or

reduced exploratory behaviour, increased frequency of urination and defecation, decreased food intake, longer time before leaving a safe hiding place, increased heart and breathing rate, less salivation, stomach ulcer, increased alertness and agility, licking of the own body and flight intention (Gregory, 2004; Sandem et al., 2004a; Sandem et al., 2004b; Davis, 1992). Additionally in sheep and cattle the time to approach an unknown object, times without moving, frequency of head rising or delay during feeding can increase (Boissy and Bouissou, 1995; Rushen, 1986).

During the slaughter process a variety of signs of fear can be observed, ranging from obvious restlessness and flight attempts with eyes wide open to simply a paralysed animal with slightly trembling nostrils, which might be licking its lips frequently.

2.3 Distress

Distress is defined in the Guidelines for the Recognition and Assessment of Pain in Animals (UPAW 1989), as a state where the animal has to devote substantial effort or resources to the adaptive response to challenges emanating from the environmental situation. Stimuli potentially leading to distress are thus more or less extreme values or levels of the various factors constituting the animal's environment. Discomfort is looked upon as a mild form of distress. All three terms, pain, distress and suffering are used in European legislative systems. In laboratory animals there are also attempts to classify pain and distress into mild, moderate and substantial (Baumans et al., 1994).

2.4 Suffering

Suffering is an unpleasant state of mind that disrupts the quality of life. It is the mental state associated with unpleasant experiences such as pain, malaise, distress, injury and emotional numbness (e.g. extreme boredom). It can develop from a wide range of causes. For example, it can occur when there is misery during exposure to cold, with the sense of fatigue and depression during cancer and when there is unremitting pain from chronic headache (EFSA, 2005).

The European Laboratory Animal Science Associations (FELASA) describes "suffering" as a specific state of 'mind', which is not identical to, but might be a consequence of, pain or distress, which may result in suffering if they are of sufficient intensity or duration, or both. Suffering is reached when pain or distress is no longer tolerable to the individual animal. Physical pain has then reached a level beyond the pain tolerance threshold, or distress has passed the level that the animal is able to cope with. Symptoms of suffering depend highly on the cause of suffering, the individual and the circumstances. Most of the symptoms of pain and fear can also be listed for suffering (Baumans et al., 1994).

2.5 Stress

Stress is physiological disturbance, which is closely linked to the mental states mentioned above which is imposed by a stressor, such as a threatening or harmful situation. Stress involves the activation of the hypothalamic-pituitary-adrenal (HPA)-axis and the activation of the sympathetic nervous system (SNS). Activation of the HPA-axis or the sympathoadrenomedullary nervous system leads to increases in heart rate and blood pressure, defecation, suppression of exploratory behaviour, reduced feeding, disruption of reproductive behaviour, exaggerated acoustic startle response, enhanced fright-induced freezing and fighting behaviour and enhanced fear conditioning. The HPA-axis is also activated by trauma and pain (Hellyer et al., 2007; Gregory, 2004).

The SNS is part of the autonomic nervous system which is controlled by certain nuclei in the brain, supplying signals to the sympathetic neurones, which prepare the individual metabolically for the muscular efforts involved in defence and flight. Responses include mobilisation of glycogen and free fatty acids, dilatation of pupils, increased heart rate and contractility and vasoconstriction in those body regions not directly involved in flight or fight mechanisms. Both pathways (HPA and SNS) are interacting, activation of one system can be associated with activation of the other, depending on the stimulus (Gregory, 2004).

2.6   Consciousness and unconsciousness

If an animal is conscious or if it regains consciousness pain, fear, and distress and consequent suffering are of special importance. For slaughter after stunning this will be relevant in cases where an animal regains consciousness before death occurs due to exsanguination, if the stunning effect does not last sufficiently long. During slaughter without stunning the animal can be subjected to pain and distress during the time until consciousness is finally lost.

For the Dialrel project "unconsciousness" is defined in a similar way to that used by anaesthesiologists: "Unconsciousness is a state of unawareness (loss of consciousness) in which there is temporary or permanent disruption to brain function. As a consequence the individual is unable to respond to normal stimuli, including pain." Consciousness is a state of awareness, which requires the function of the brainstem and projections in the relevant cortical regions. Following (Zeman, 2001) in everyday neurological practice consciousness is generally equated with the waking state and the abilities to perceive, interact and communicate with the environment and with others. As a matter of degree a range of consciousness states extend from waking through sleep until unconsciousness is reached. Furthermore there is no distinct boundary and drifting in and out of consciousness is possible. Structures in the upper brainstem core, play a critical role in arousal and thalamic and cortical activity supply much of the "content of consciousness" (Zeman, 2001). Butler and Cotterill (2006) suggest that the neural substrate for complex cognitive functions that are associated with higher-level consciousness are based on patterns of neural circuitry and re-entrant loops. Reviewing brain structures in mammals and birds the authors found, that many of the major pathways and circuits present in mammalian brains and identified by various workers as crucially involved in the generation and maintenance of consciousness are also present in avian brains. These neuroanatomical equivalents include the cerebrum (cortex and subcortical nuclei) and the interbrain (e.g. thalamic nuclei). As shared neural circuits do not, in and of themselves, reveal whether birds are conscious, the authors additionally refer to behavioural evidence for higher cognitive abilities of birds (Butler and Cotterill, 2006).

If the respective brain structures do not function, consciousness will be lost. Loss of consciousness or regaining of consciousness must be seen as a process, which depending on the slaughter method used may take some time (see below).

Accordingly signs of consciousness are variable and setting standards for diagnosis of consciousness/ unconsciousness must depend on the slaughter method applied and the way in which it is applied. Regaining consciousness after stunning can happen quickly, depending on the stunning method. For example after gas stunning, chickens can become completely awake only a very few seconds after having shown the first activities signifying a functioning brain stem (regular breathing and positive corneal reflex). Regular breathing should be taken as an alarm signal with regard to assuring good stunning effectiveness and timely effective bleeding (Wenzlawowicz and Holleben, 2005).

2.6.1 Assessment of consciousness / unconsciousness

It is generally agreed in the context of anaesthesia and slaughter, that physical collapse and the lacking of goal directed movements are important signs with regard to evaluation of consciousness. In the conscious animal the cerebral cortex integrates both functions (posture and movement). Therefore physical collapse can indicate that the cortex is no longer able to control postural stability (Muir, 2007). However an animal that had already collapsed after a dramatic loss of blood pressure may nevertheless regain consciousness due to the body's own counter-regulation mechanisms. Thus physical collapse must not be a definite sign of loss of consciousness but is an indicator of an early phase in the progression towards overall unconsciousness. Animals can drift in and out of consciousness as they lose or regain it (Gregory, 2005a).

The cortex is not always involved in the maintenance of standing posture or basic propulsive movements. However, its participation is needed to control postural stability and closely coordinated movements. Postural control to avoid physical collapse and goal directed movements are regulated both in the spinal cord by autonomous reflexes and by supraspinal commands, at all levels of the motor control hierarchy. Perturbations of simple programs initiate strategic and motor programming at higher motor levels involving cerebellum, basal ganglia, and cerebral cortex by means of anticipatory (feedforward) motor responses (Grillner et al., 2008; Lalonde and Strazielle, 2007; Deliagina and Orlovsky, 2002).

After slaughter, consciousness may be indicated by movements like standing up again, righting and looking around. Other movements are more difficult to explain, because they also can be due to the effect of stunning (clonic phase after captive bolt or electric stunning). In Addition they can also be a result of lost function of the cortex, which normally provides control over autonomous movements. Finally it is very difficult do standardize descriptions like "purposeful" or "coordinated" movements (Grillner et al., 2008; Jennings, 2004). For evaluation of movements in the context of consciousness – as for all signs - it is necessary to take other signs into account as well (Holleben, 2009). However collapse occurring when a freely standing animal falls to the ground is the earliest indication of approaching insensibility after the neck cut (Gregory et al., 2010; Grandin, 1994a; Blackmore, 1984).

Different cognitive responses have been assessed after puntilla slaughter (neck stab) in Bolivian cattle in order to evaluate cranial nerve responses, and which parts of the spinal cord was still intact immediately after the animals were ejected from the pen. The responses were: 1. Reaction to a threat stimulus, which was done by rushing the hand towards the eyes and observing if the animal reacted by closing its eyes or backward head movement. 2. Response to sudden noise stimulus of clapping the hands up to 5 centimetres from the animal's ear and observing an ear movement and alerting response. 3. Response to air been blown on the noise, which when positive was reported as a backward movement of the head. 4. Responses to different odours or flavours when introducing a stick in front of the nostril or in the mouth, which when positive was reported as nostrils flaring and/or tongue movement. 5. Localised skin response, stimulated by a single needle stimulus in the skin over the frontal bone (Limon et al., 2010). The authors concluded that over 70% of the animals were sensible, based on a high percentage of positive responses to threat, flavours, noise stimuli and needle skin stimulation (Limon et al., 2010). The cognitive threat test had a response frequency of 61% implying, that this test may be useful in assessing consciousness of animals after slaughter without stunning. This is provided that the animal is able to focus on the test stimuli and is not distracted by other events.

Clinical indicators of general anaesthesia (Muir, 2007; Teasdale and Jennett, 1974) can be used to assess insensibility and unconsciousness as long as the slaughter method itself does not change or mask the clinical signs. For example, during the epileptic fit immediately after

electrical stunning, reflex testing cannot be accessed because of hyperactivity, caused by the stunning method itself. Another example is cranial nerve reflexes. The function of the cranial nerves included in the reflex can be directly affected by the stunning methods (e.g. mechanical stunning producing cranial nerve concussion or electrical stunning electrodes placed near the orbits). In many cases restraining methods or suspension from a shackle on the line limit the movements and physical responses. Consequently the appropriate cranial nerve reflexes and reflex testing should be always used in light of the stunning/slaughter method and restraint system.

Responses are wilful movements of the body or parts of the body, which cannot occur without involvement of the somatosensory, nociceptive, auditory, olfactory, gustatory or visual cortex. Whereas reflexes are defined as involuntary, purposeful, and orderly responses to a stimulus involving integration in the spinal cord or brainstem, which may be linked to perception.

Reflexes especially those including the cranial nerves are nevertheless helpful to assess brain function, this is because the cranial nerves enter the brain above the level of the spinal cord. Therefore a positive cranial nerve reflex is not complicated by spinal cord severance or injury and cannot be interpreted as a "spinal cord reflex". If a cranial nerve reflex is positive, the pathway that the cranial nerve reflex takes through the brain is still functional. Cranial nerve reflexes assist in getting an overall picture of brain dysfunction. If all negative, they are good indicators of impaired midbrain or brainstem activity and unconsciousness can be inferred, provided the muscles and afferent and efferent nerves which execute the response are still capable of working and not preoccupied with other stimuli (Gregory, 1998a).

Table 1: assessment of consciousness or unconsciousness (*signs relying on functioning cranial nerves can only be evaluated if nerve function is not directly affected by the stunning or slaughter method)

Signs *	Physiological implication	Comments
Eye reflex (touching the cornea or the lid, eye lids close)	Corneal reflex is a brain stem reflex, **its *absence* indicates loss of brain stem function and thus loss of consciousness.**	Positive eye reflexes alone do not indicate consciousness but can be taken as a sign that the brain is reorganizing e.g. after stunning. Positive reflex responses may be present for several minutes after the cut in unconscious animals (Blackmore, 1984). After effective captive bolt stunning eye reflexes must be absent.
Wide open relaxed eye and pupil	The cranial nerves innervating the eyeball, pupil and the lid do not function and thus brain activity is impaired. **A wide open relaxed eye** with a blank stare can be taken as an **additional sign for unconsciousness**.	Wide open relaxed eyes and pupils often occur in dead animals. However before death this may be a transient state. Ocular signs are variable and should never replace respiratory and circulatory signs (Muir, 2007)
Blinking	Blinking is generated by an eye preservation reflex. ***Absence*** of blinking is based on lost sensory and motor qualities of the concerned cranial nerves and **is a reliable sign of anaesthesia.**	If repeated spontaneous blinking is present this may be a sign of consciousness/sensibility, especially if occurring together with eye movements, focused on external stimuli.
Nystagmus	"Flickering eyeball", indicates dysfunction in the hindbrain if not triggered by other stimuli. **The implication of nystagmus depends on the slaughter method.**	Nystagmus is often seen during the epileptic fit together with effective electrical stunning. After captive bolt stunning insensibility may be questionable if the eyes are rolled back or vibrating.
Focused eye movements	Involves cortical activity in perception and goal directed motor activity of eyeball muscles (Grillner et al., 2008); **if *present* animal is conscious.**	Eye follows stimuli from surrounding movements (eye tracking of movements).
Cognitive threat test	Involves cortical activity in perception, coordinated motor activity of cranial nerves and for moving back of the head motor cortex activity; **if *positive* consciousness is highly likely**.	Threat stimulus by rushing the hand towards the eyes led to animals reacting by closing its eyes and some also by moving the head backwards (Limon et al., 2010),

Signs *	Physiological implication	Comments
Gasping	*Gasping* (single irregular mouth or beak opening mostly without ventilation of the lungs) is a sign of a dying brain and **does not indicate consciousness.**	Gasping may be the first sign, that the brain is reorganizing after stunning. A twitching nose (like a rabbit) may be a sign of partial sensibility. Gasping after gas stunning may lead to recovery.
Rhythmic breathing	Rhythmic breathing is coordinated by the brainstem. *Absence* of rhythmic breathing **indicates lost consciousness.**	Rhythmic breathing alone does not indicate consciousness but can be taken as a sign that the brain is reorganizing e.g. after stunning. Breathing may be present for several minutes after the cut in unconscious animals (Blackmore, 1984).
Vocalization	Requires function of somatosensory and motor cortex; *Vocalization* **indicates consciousness.**	Monotonous sounding "false vocalization" can occur in synchrony with breathing movements and spasms in the unconscious state. After throat cut the larynx is severed from the trachea, vocalization is no more possible. Noises generated by fluids bubbling and gurgling in the trachea may be falsely taken as vocalization.
Kicking	May be a sign that inhibition of spinal patterns is lost. *Kicking* does **not necessarily indicate consciousness.**	Kicking may be a sign of effective stunning (electrical or mechanical stunning), it may occur in unconscious animals (gas stunning) or during/ after severance of the spinal cord or at the end of bleeding. Following captive bolt stunning its onset can coincide with the development of an isoelectric EEG.
Righting/ arched back	Righting reflex/ response may be helped by subcortical CNS structures, but in most cases means function of the cerebral cortex and return of proprioception and muscletone. **If *present* it is very likely that the animal is sensible.**	Righting may be impaired by shackling or restraint, freezing behaviour or the use of certain current forms in electrical stunning. A relaxed tail does not occur together with an arched back or righting.
Floppy head	A floppy relaxed head and neck, e.g. hanging down in shackled animals indicates that muscle tone and in most case cerebral control over posture are lost. If *present* in most **cases consciousness is lost**.	Some current forms can have a very relaxing or immobilizing effect, e.g. in poultry. In these cases signs of reawakening after stunning may be completely masked. The absence of a tonic spasm after captive bolt stunning is a sign of a low depth of concussion, and so in this case a floppy head and neck contraindicate a good stun.
Wing flapping	**Wing flapping** may be a sign that inhibition of spinal patterns is lost, but also can mean coordinated goal directed flight attempts. It **often indicates consciousness**.	If wing flapping on the rail is expressed together with vocalization and breathing, the bird is showing escape behaviour and is conscious. Unconscious wing flapping occurs during head-only electrical stunning, concussion stunning, CAS stunning or at the end of bleeding.
Nose pinch	**Response to nose pinch** indicates activity of the respective circuit of sensory and motor cranial nerves and **indicates possible return to sensibility.**	If positive, pinching into the nasal septum is followed by pain reaction/ withdrawal. It is a helpful tool in shackled animals, which are immobilized by their position. After electrical stunning consciousness may be recovered before sensibility to pain.
Tongue hanging out	**A relaxed tongue** may indicate loss of cranial nerve function but **is not a reliable sign of unconsciousness. A curled tongue** may be a sign of **possible return to sensibility.**	The tongue may hang out also due to gravity when the jaw muscles are relaxed, and this is a sign that the animal is unconscious. This can be confirmed by manipulating the jaws by hand and if there is no resistance to movement, the animal is unconscious. After neck cut the tongue may hang out because the respective nerves and muscles are cut.

This table by Adams and Sheridan (2008) is based on an article of Temple Grandin http://www.grandin.com/humane/insensibility.html, signs of effective stunning were taken from the EFSA report http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620775454.htm and modified by the authors' experience;

2.6.2   Measurement and interpretation of brain electric activity

The indicators mentioned above can be supported under experimental situations by measurements of electroencephalography (EEG) and electrocorticography (ECoG). These are widely used to record the brain electrical activity to determine the state of consciousness and brain disorders in humans and animals. Absence of electrical activity or a certain level or rhythm of electric activity or absence of somatosensory, auditory or visually evoked responses may indicate that the animal is dead or unconscious. However as evoked electrical activity of the brain exists as well in anaesthetised animals, it is difficult to predict only from EEG or ECoG, if the animals is really conscious. Nevertheless, evoked responses have been very helpful in giving comparative assessments of different stunning or slaughtering methods. Auditory evoked potentials have been suggested to be a more precise indicator of the level of consciousness than the EEG after CO_2 stunning of pigs (Rodriguez et al., 2008; Martoft et al., 2001).

According to (EFSA, 2004, page 30) it is suggested that in spite of the differences in the way stunning methods induce unconsciousness, an animal can be judged to be unconscious and insensible if EEG shows changes that are incompatible with consciousness, e.g. grand mal epilepsy, or a prolonged quiescent period with less than 10% of the pre-stun EEG power content or abolition of evoked electrical activity in the brain. The abolition of evoked potentials has been used as an objective and unequivocal indicator of loss of brain responsiveness and hence, loss of consciousness, in many species. However, the presence of evoked potentials does not necessarily indicate consciousness, because visual evoked potentials can be present in anaesthetised animals and when the EEG is isoelectric, especially in poultry (EFSA, 2004; Zeman, 2001; Gregory, 1998c).

Besides their role in determination of the level of consciousness, changes in the power spectra of the EEG have been shown to reflect alterations in the activity of the cerebral cortex associated with perception of acute pain in humans (Chen et al., 1989) and animals, e.g. surgery, castration, tail docking and mulesing. Although being indirect measures of pain, spectral changes reflect cortical activity and hence are likely to reflect the cognitive perception and processing of noxious stimuli (Barnett, 1997). Recently the EEG and a minimal anaesthesia model has been validated for the assessment of noxious sensory input in cattle (Gibson et al., 2007).

2.7   Death

The definition of death, chosen for the Diarel project is the same as that used by EFSA (2004, page 15): "Death is a physiological state of an animal, where respiration and blood circulation have ceased as the respiratory and circulatory brain centres in the Medulla Oblongata are irreversibly inactive. Due to the permanent absence of nutrients and oxygen in the brain, consciousness is irreversibly lost. In the context of application of stunning and stun/kill methods, the main clinical signs seen are permanent absence of respiration (and also absence of gagging), absence of pulse and absence of corneal and palpebral reflex."

It is important to look at death as a process with different interdependent functions. For example, if the function of the brainstem is sufficiently impaired, respiration will cease. The brainstem is essential for breathing. It is also responsible for the full functionality of the cortex (see 2.6). Thus brainstem death or sufficient damage also leads to the irreversible loss of consciousness. The heart is powered by its own autonomous mechanism. After respiration has ceased the heart will continue to function as long as enough oxygen and energy are available and the waste products can be sufficiently cleared. If cardiac death or sufficient cardiac dysfunction occurs before brain dysfunction, cerebral perfusion will be reduced or stop resulting in the loss of supply of energy and oxygen to neurons within the brain and

accumulation of waste products. This causes brain dysfunction and brain death. Correct slaughter will lead to rapid effective blood loss. Consequently energy and oxygen supply progressively falls to the heart and brain and both will stop to function over time (Michiels, 2004; Rosen, 2004; Pallis, 1982a; Pallis, 1982b; Pallis, 1982c; Pallis, 1982d).

2.8   Physiology of exsanguination or bleeding out

Slaughter is the process of bleeding to induce death, usually by severing major blood vessels supplying oxygenated blood to the brain (see Dialrel glossary in the annex of this report and EFSA (2004)). After severing the major blood vessels of the neck, with either reversible or without stunning, animals die due to loss of circulating blood volume and the resultant cerebral anoxia. Exsanguination can be carried out either by neck cut or thoracic cut.

A neck cut according to the Dialrel glossary, involves severing of major blood vessels in the ventral neck region (skin and vessels cut simultaneously). Neck cutting also referred to as throat cutting means an incision below the angle of the jaw. The two carotid arteries and jugular veins are severed simultaneously with the oesophagus trachea and vagus nerves. This practice has been suggested as not been optimal with regard to hygiene reasons. According to the EU hygiene regulations, "the trachea and oesophagus must remain intact during bleeding" (VO EG Nr 853/2004, Annex III, Sec I, Chap IV, No. 7. a[3]). Nevertheless the practise of severing the trachea and oesophagus is explicitly allowed in the EU hygiene regulations in the case of religious slaughter.

The thoracic cut according to the Dialrel glossary is described as "severing major blood vessels emerging from the heart by inserting a knife in front of the brisket or sternum (double cut: first the skin, then, with another knife, the vessels)". By thoracic or rather pre-thoracic cut of cattle (also imprecisely referred to as chest stick), the brachiocephalic trunk is severed immediately cranial to the thoracic inlet. The brachiocephalic trunk is a single large vessel that emerges from the aorta and gives rise to the common carotid arteries, which supply the head with blood.

2.8.1   Loss of blood volume, loss of blood pressure

The circulating blood volume in animals is estimated to be 8% of body weight and about 18% of total cardiac output flows through the brain at any one time (EFSA, 2004, page 23). With adequate incision of the neck vessels all animals loose between 40 and 60% of their total blood volume and the pattern and rate of loss is similar in the various species examined (Warriss and Wilkins, 1987). Cutting leads to a drop in blood pressure, which may result in hemodynamic instability, interruption of blood supply to the brain and other organs. This can result in insufficient perfusion of tissues with blood, leading to inadequate oxygenation and removal of toxic waste products. Life threatening drops in blood pressure are often associated with a state of shock – a condition in which tissue perfusion is not capable of sustaining aerobic metabolism. The bodies compensatory response to a hemorrhagic shock caused by bleeding, includes systemic reactions such as increased heart rate, local vasoconstriction of arterioles and muscular arteries and shifting of extravascular and venous reserve fluids to the circulating blood volume. This response aims to enhanced cardiac output and maintenance of perfusion pressure, especially in heart, brain and adrenal glands (Guiterrez et al., 2008). The time lag between severe haemorrhage and unconsciousness certainly depends on whether and how long compensatory mechanisms are successful or whether they are eventually overwhelmed by blood volume losses (Gregory, 2005a).

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[3] 7. Stunning, bleeding, skinning, evisceration and other dressing must be carried out without undue delay and in a manner that avoids contaminating the meat. In particular:
(a) the trachea and oesophagus must remain intact during bleeding, except in the case of slaughter according to a religious custom;

The immediate loss of blood pressure after neck cutting has been often described as being important for the rapid loss of consciousness (Rosen, 2004; Levinger, 1995; Levinger, 1976; Levinger, 1961). Mechanisms may be ischemia as well as changes in cerebrospinal fluid pressure (Rosen, 2004; Levinger, 1976; Lieben, 1926). Rosen (2004) suggested that following Shechita the collapse in jugular venous pressure, without replacement with carotid blood would result in impaired maintained of brain structure. Based on recent research, there is no histological evidence that the sudden decompression of the cranial vault affects the brain structure and function (Gibson et al., 2009b).

However loss of consciousness may not be permanent, as transient blood pressure rises together with a resurgence of consciousness have been shown in monkeys with severe haemorrhagic shock by Bar-Joseph et al. (1989).

A literature review by Gregory (2005a) presumes that in mammals such as men monkeys, dogs and rats consciousness is lost if 30-40 % of the total blood volume is lost or if blood pressure drops to below values between 35 and 50 mmHg. From this review the author also concludes that respiratory distress can occur during slow haemorrhage. Blood pressure loss can be very disturbing to humans (Hamlin and Stokhof, 2004) and probably to animals of other species (EFSA, 2004, page 23).

In humans the effect of haemorrhage has been classified from I to IV. Central nervous system symptoms are "Normal" (class I, blood loss: < 15%), "Anxious" (class II, blood loss: 15-30%), "Confused" (class III, blood loss: 30-40%) and "Lethargic" (Class IV, blood loss: >40%), class I being a non shock state, class IV a pre terminal event requiring immediate therapy. Irreversible hypovolaemic shock and the moribund comatose state result from a loss of more than 50% of the circulating blood volume (Guiterrez et al., 2008).

As said above not only the percentage but also the time during which blood volume is lost should be considered. Table 2 shows the results of some investigations concerning blood loss after neck cutting without stunning.

Table 2: Blood loss in cattle and sheep by time after neck cutting without stunning

Time after cut (s)	Sahlstedt (1929) cited in Levinger (1995; 1976)		Anil et al. (2006)	Anil et al. (2004)
	Percentage of total blood loss CATTLE	*Percentage of **total blood volume*** *CATTLE*	Blood loss as a percentage of blood lost *at 120 s CATTLE*	Blood loss as a percentage of blood lost *at 90 s SHEEP*
5.7				25
14.1				50
17.3			25	
30	33	*17*		
31.8				75
37.5			50	
55.8				90
60	50	*25*		
68.0			75	
94.4			95	
120	***70***	***35***		
180	***83***	***42***		
240	90	*45*		
300	95	*48*		

*As approx. 50% of the total blood volume is lost during bleeding the percentage of total blood volume is calculated percentage of total blood loss divided by two.

The results from Levinger indicate that loss of 30% of total blood volume might be reached in cattle at about 60 to 90 seconds after the start of blood flow from the cut neck (a loss of 30 to

40 % of the total blood volume was associated with the loss of consciousness (Gregory, 2005a)). While in sheep this point is reached earlier. Meat chickens slaughtered by Shechita lose about 40 % of their total blood volume within 30 seconds of neck cutting (Barnett et al., 2007).

The critical values of blood pressure can be reached early in sheep (5 to 6 seconds) (Levinger, 1976). But the blood pressure loss can vary widely between individual animals. Lieben (1928; 1925) measured the blood pressure in the arteria vertebralis, arteria carotis and aorta after a cut performed by a professional Jewish slaughterman in 4 goats, 4 sheep, 5 calves and one young bull. He found rapid blood pressure drops in most of the animals. The blood pressure in the aorta decreased more slowly than pressure in the arteria vertebralis (it is not always possible to tell the exact time in seconds from the graphs presented – estimated time in seconds for blood pressure drop is between 2 and 10 seconds post cut). The author described that in one of the 12 animals, (which was not presented in the graphs), that blood pressure in the aorta rose for about 27 seconds and only then slowly decreased over three minutes to zero (no measurements in the a. vertebralis were performed). In another sheep a rise of blood pressure after the initial fall could have been triggered by pressing a cloth into the wound. In one calf the rise in blood pressure, also after an initial fall, could have been triggered by the spreading of paper in front of the wound to shield the operating room against the strongly sputtering blood. The spreading of the surgical paper caused a heavily sizzling noise and happened between 2 and 3 seconds after the cut. In another animal a bad cut was intentionally performed with a blunt knife torn back and forward for 10 seconds on an unstretched neck. Which resulted in an initial rise in blood pressure in both arteries for 7 seconds and afterwards a decrease below the starting point, after 35 seconds blood pressure increased and then further undulation (Lieben, 1925). Thus not all cases of delayed blood pressure loss could be explained by bad performance of the cut.

Newhook and Blackmore (1982b) also found an increase in femoral blood pressure in three conscious sheep which reached its maximum within 6 to 7 seconds after the cut and stayed high for 10 to 20 seconds (in one sheep there was no increase but the blood pressure remained constant for 3.6 seconds). Whereas in 5 sheep under barbiturate anaesthesia blood pressure decreased immediately after the blood vessels were severed. In anaesthetised calves femoral blood pressure dropped below 40 mmHg 20 seconds after ventral neck cut. In this study no signs of occlusions were seen neither in the cephalic or cardiac vessel ends due to the fact that animals were heparinised. However occlusions of the arteries lead to recovery episodes in blood pressure and the blood pressure fell sooner when no occlusion of the arteries occurred (Anil et al., 1995b).

To summarize loss in blood pressure cannot be generally taken as immediate and rapid but variations between species and individual animals exist.

2.8.2   Cerebral perfusion after neck cutting

The blood supply to the brain of ruminants is derived by a vascular network, the "rete mirabilis occipitale". The rete mirabilis is supplied as well by branches diverging off the carotid artery as by the vertebral artery. It is more extensive in cattle than in sheep. Whereas in goats there are less evident connections between the anastomosis of the two vessels and the rete (Baldwin and Bell, 1963a; Baldwin and Bell, 1963b; Levinger, 1961). Vertebral arteries are also present in poultry (Mead, 2004).

Blackman et al. (1986) found that sequential boli of dye, infused into the heart of two 1-10 day old anaesthetized calves after bilateral severance of the common carotid arteries and jugular veins, could be detected passing through the cerebral vessels for more than 100 seconds after the cut. The passage of dye through cerebral vessels could not be observed in

the cerebrum of the 2 adult sheep after bilateral severance of major blood vessels. In sheep when the vessels were severed on one side of the neck only, the passage of dye was noted for at least 53 seconds. The authors concluded that there are major differences between sheep and calves in the blood supply to the brain due to the vertebral arteries in cattle. The vertebral arteries of cattle are not severed by the neck cut due to their passage close to the spinal cord. Unlike sheep, the vertebral arteries in cattle are capable of maintaining the cerebral blood flow. This effect is supposed to be even stronger in unanesthetised animals, because anaesthesia is known to reduce cerebral blood flow (Blackman et al., 1986). Levinger (1961) concludes from similar experiments that the cerebral blood flow through the vertebral arteries would not be sufficient to supply the brain. Nevertheless, even if the blood flow from the vertebral arteries may not be sufficient to supply the whole brain, it is likely that it contributes to prolong brain function and consciousness.

Anil et al. (1995a) found that, in electrically stunned calves suspended upside down by a hindleg, carotid occlusion delayed the time to isoelectric ECoG (brain failure). The mean arterial blood pressure was held for longer when carotid occlusion occurred and vertebral artery blood flow could be maintained at about 30% of its initial level for up to 3 minutes. In some animals vertebral artery blood flow increased substantially following sticking.

Shaw et al. (1990) ligated the vertebral arteries in 4 out of 8 calves and measured the onset of brain failure by EcoG. They concluded that factors other than blood flow from the vertebral arteries contribute to the prolonged time to loss of electrocortical activity after slaughter observed in calves.

Bager et al. (1988) looked at cerebral blood flow by measuring the venous blood leaving the head ends of the jugular veins in calves, and suggested that factors impeding the retrograde blood flow from the brain and thus rising cerebral blood pressure might be important.

Daly et al. (1988) suggested two explanations: first there are differences between animals in the proportion of the total cerebral blood flow which is contributed by the vertebral arteries. Secondly the amount of blood reaching the brain via the vertebral arteries after slaughter is very close to the minimum blood flow necessary to sustain electrical activity in the brain cortex, so that slight differences in individuals would result in large variations (Daly et al., 1988).

It is important to note that the above mentioned experiments have been conducted with only limited numbers of animals and already important individual as well as species differences have been found (Levinger, 1961). In small ruminants Levinger (1961) found that animals collapsed but were able to regain posture when the carotid arteries were clamped, whereas loss of posture was definite when the collateral pathways via vertebral and occipital arteries were also blocked. Even in sheep, where the vertebral arteries pathway to the brain is usually stated to be of minor importance, this route could be found in some animals and activated in others (Nangeroni and Kennet, 1963).

Cerebral hemodynamic compensatory mechanisms will also help to maintain brain function during reduced systemic blood pressure. Cerebral perfusion pressure (CPP), the driving force for blood through the cerebral circulation is defined as the difference between mean arterial pressure and venous backpressure or intracranial pressure. As CPP falls, cerebral blood flow is initially maintained by vasodilation of resistance arterioles, a reflex known as autoregulation. With further reductions in CPP, the autoregulatory capacity is exhausted and cerebral blood flow falls as a function of pressure, but increases in oxygen extraction fraction will maintain cerebral oxygen metabolism and tissue function up to a point (Derdeyn, 2001).

To summarize factors influencing the dynamics of cerebral blood flow after neck cutting seem to be very complex and individual differences as well as age, weight and breed have an

impact so that the picture given by the above named investigations on cerebral blood perfusion is still incomplete with regard to explaining prolonged consciousness after the cut.

2.8.3 Impacts on bleed-out or exsanguination

To understand the impacts on the time to loss of consciousness it is important to look at the factors that influence bleeding. Gregory (2005b) gives an overview on the factors affecting bleeding, which are further explained below:
- Blood vessels that are severed;
- State and patency of the sticking wound;
- Cardiac arrest at stunning;
- Orientation of the carcass;
- Vasodilatation and vasoconstriction in the capillary bed;
- Tonic muscle contraction squeezing blood capillaries and vessels and
- Clonic activity causing movements of blood towards the sticking wound.

In sheep, bleeding out by cutting both the common carotid arteries and the jugular veins is the quickest method of abolishing brain responsiveness (loss of visual evoked responses, relevant EEG changes) compared to cutting only one carotid artery, only the jugular veins or cardiac ventricular fibrillation (Gregory and Wotton, 1984a; Newhook and Blackmore, 1982b).

In captive bolt stunned calves, which were either cut by thoracic cut, Halal "high" neck cut, or Halal "low" neck cut (in the brisket region), highest blood loss after 60 seconds as percent of live weight occurred with the thoracic cut, followed closely by Halal "low", Halal "high" and finally unilateral cut (Gregory et al., 1988a). The length of the sticking wound in the skin has been found to be important in electrical stunned pigs (Anil et al., 2000).

In poultry cutting both carotid arteries, compared to cutting one common carotid artery and/ or one jugular vein, induced impaired brain function most rapidly (Gregory and Wotton, 1986).

The quality of the cut including sharpness of the knife and capability to perform a swift uninterrupted cut within a very short time is often mentioned especially in the context of Shechita (Rosen, 2004; Lieben, 1925). This could be partly responsible for further impacts like vasoconstriction, clotting, ballooning or false aneurysms (Gregory et al., 2006; Anil et al., 1995a; Anil et al., 1995b; Gregory et al., 1988a; Graham and Keatinge, 1974), which may lead to occlusions of the severed ends of the carotid arteries. Occlusion of the carotids has been shown to prolong markedly the time to loss of ability to stand or to attempt to rise in calves (Blackmore, 1984). Gregory et al. (2008) found a prevalence of large (>3 cm outer diameter) false aneurysms in cattle carotid arteries of 10 percent for both Shechita and Halal slaughter. The prevalence of animals with bilateral false aneurysms was 7 and 8 percent for Shechita and Halal slaughter, respectively, whereas no false aneurysms occurred during bleeding in cattle that were electrically stunned and simultaneously developed a cardiac arrest. The authors concluded that combination of false aneurysms and collateral routes to the brain present a risk of sustained consciousness during religious slaughter in cattle.

In a recent study, the time to physical collapse was examined in 174 adult cattle which were restrained in the upright position and then released immediately from the restraint following the Halal cut. The frequencies of swelling and false aneurysm in the cephalic and cardiac severed ends of the arteries were recorded in relation to time to final collapse. Delays in collapse were associated with swelling of the cephalic and cardiac ends of the carotid arteries (Gregory et al., 2010).

Another impact on the patency of the carotid arteries is collapse of the vessels by pressure of the surrounding tissue. Following the cut the severed ends may retract below the wound

surface, so that they are covered by surrounding muscle tissue. Because carotids and trachea are linked by connective tissue, respiratory movements can cause the backward movement of the trachea within the thoracic cavity, this may further cause disruption of blood loss from the carotids. Certain positions of the animal during bleeding may facilitate this effect (Levinger, 1995; Anil et al., 1995a; Hoffmann, 1900). Rosen (2004) mentioned the importance of correct post cut restraint with regard to correct bleed-out and time to loss of consciousness.

It has been suggested in cattle that when inverted bleeding might be impaired. This is suggested as been the result of the weight of the abdominal organs pressing on the diaphragm and major veins. The added pressure on the heart may decrease stroke volume (compare "cardiac tamponade") and the pressure on the veins may impair venous reflux (Adams and Sheridan, 2008). In one study, cardiac output in cows in dorsal recumbency, that were not bleed, changed only after 30 minutes in which case the impact on venous return may be small or negligible (Wagner et al., 1990).

When discussing stunning in relation to bleed-out it is often debated whether bleed-out rates and total blood loss resulting from neck cutting without stunning are higher than those with stunning. Anil et al (2006; 2004) investigated exsanguination of sheep after electrical stunning, captive bolt stunning and slaughter without stunning and also exsanguination of cattle after captive bolt stunning and slaughter without stunning. They found no difference and concluded that bleed-out was not adversely affected by stunning nor improved by neck cut without stunning. This was confirmed by Gomes Neves et al. (2009), who assessed bleeding efficiency after captive bolt stunning and after slaughter without stunning in 171 steers through analysis of residual haemoglobin content in the longissimus colli muscle. Velarde et al. (2003) showed that lambs that were hoisted and bled without being stunned, lost less blood from the sticking wound than lambs that were electrically stunned (250 V, 50 Hz, 3 s), hoisted and then stuck. The authors mentioned as a likely explanation that the muscle contractions associated with electrical stunning forced blood away from skeletal muscles towards the vessels in the thorax and abdomen.

Haemoglobin content in different muscles – indicating quality of bleed-out – did not differ in sheep and calves that were subjected to captive bolt stunning or Shechita (Kallweit et al., 1989). In broiler chicken no difference was found after different slaughter methods including electrical stunning and kosher slaughter in the amount of blood loss after neck cutting and in the blood retained in different cuts (Kotula and Helbacka, 1966a; Kotula and Helbacka, 1966b).

In captive bolt stunned animals the longer the time interval between stunning and sticking, the less blood is lost, but the effect is less than often assumed (Blackmore and Delany, 1988; Vimini et al., 1983). Even 6 hours after delayed bleeding in cattle stunned by captive bolt and subsequently killed by cardiac arrest and then shackled, higher residual blood levels were only found in the forequarter muscles. Although there was a large decrease in the amount of blood flowing from the sticking wound, when sticking was delayed, the effects on carcass appearance and residual haemoglobin in the muscle were small (Gregory et al., 1988b).

It is often suggested that cardiac arrest will decrease bleeding rate. In pigs a functioning heart does not appear to be necessary for adequate exsanguination (Warriss and Wotton, 1981). In sheep there might be an influence (Gregory and Wilkins, 1984), however effects on bleed-out seems to be due more to the method of sticking rather than the beating heart (Warriss and Wilkins, 1987). Vimini et al. (1983) investigated delayed bleeding 3, 6 and 15 minutes after captive bolt stunning and found that not the heart, but muscle contraction, time of bleeding and gravity were important. Most of the blood was still lost after the heart had already stopped beating. In broiler chickens cardiac arrest resulted in a slower initial rate of bleeding but by 2.25 minutes after neck cutting there was no effect on the amount of blood collected by

different neck cutting method including ventral and dorsolateral cuts performed by automatic neck cutters (Gregory and Wilkins, 1989a).

The effects of animals position on bleeding rate may have been previously overestimated, e.g. in sheep bleeding is slightly more rapid in a recumbent position than if suspended in a vertical position (Blackmore and Delany, 1988). In cattle Hess (1968) after captive bolt stunning, recovered more blood from a hanging carcass than in a recumbent position. Another investigation comparing recumbent and hanging position after electrical stunning and hanging position after captive bolt stunning produced similar results in all methods. It has been concluded, that the capability of the person performing the cut is more important than stunning method or position of the carcass (Bucher et al., 2003).

It is possible that differences between brain size, blood volume, and arterial cross sectional area, especially with increasing body size may have an effect on the time to loss of consciousness. The carotid arteries of adult cattle may be too small relative to total blood volume to allow for sufficiently fast bleed-outs and a drastic loss in blood pressure. It is further suggested that in sheep and cattle different percentages of the total blood volume are necessary to supply the brain (Adams and Sheridan, 2008). The weight of the brain relative to the total body weight in sheep is 0.26% and for cattle (500-600 kg life weight) it is only 0.07 to 0.08% (Nickel, Schummer and Seiferle, 1984). This implies that a lower proportion of the total blood volume is necessary to perfuse the brain of cattle than it is for sheep and that cerebral perfusion will inherently be maintained for a longer period during blood loss in cattle than in sheep or goats. This argument may be supported by the fact, that due to practical aspects many studies on time to loss of consciousness have been conducted on smaller animals, e.g. sheep and calves and that these results differ from most findings under practical conditions for full grown animals. This applies as well for slaughter without stunning (Gregory et al., 2009; Gibson et al., 2009b; Bager et al., 1992; Gregory and Wotton, 1984a; Gregory and Wotton, 1984c; Lieben, 1928) as for slaughter after stunning (Wenzlawowicz, 2009; Gregory et al., 1996; Bager et al., 1992).

Finally there is also a possible role of the sympathetic nervous system, e.g. if this is activated by preslaughter stressors leading to changes in regional blood flow and slow bleeding rate. Catecholamine release by preslaughter stressors can affect the distribution of blood between the peripheral vascular beds, from where blood is shifted into the central large vessels in case of stress (Warriss and Wilkins, 1987) and consequently more blood loss is required to achieve unconsciousness. In this context the severance of the vagus nerve has to be discussed (Gregory, 2005b). Gibson et al. (2009a) found, that the drop of blood pressure following transection of the ventral neck tissue without disruption of blood circulation was immediate and more pronounced than after blood vessel transection without severing the neck tissue, which was however similar to slaughter by ventral neck cutting of intact animals (Gibson et al., 2009b; Anil et al., 2006). Gibson et al. (2009a) assumed that the effect on blood pressure by cutting the neck tissue without cutting the major blood vessels was due to the severance of the vagosympathetic trunk.

To summarize there are manifold impacts on the quality of bleeding and thus the time to loss of consciousness, some of which cannot be mitigated by the performance of the cut.

3   Principles of restraint and requirements for restraint

According to the Dialrel glossary, restraining means restricting the movement of an animal / holding the animal in a correct position, so that a procedure (e.g. sticking or stunning) can be carried out accurately. The ideal restraining method for slaughter depends on the animals to be slaughtered, the method of slaughter (including slaughter speed and the process for

stunning and/or cutting) and the capabilities of the staff. There are some basic principles of restraint with regard to animal welfare which have to be fulfilled independently from the slaughter method (Holleben, 2007):

- An animal should be able to enter / to be put in the restraining device without stress;
- restraining itself must cause as little stress / strain as possible;
- restraint time should be as short as possible;
- restraining must not cause injuries;
- when a mechanical or electrical stunning method is applied the restraining method must allow the secure positioning of stunning devices; when slaughter is performed without stunning the restraining method must allow the correct application of the bleeding cut;
- prompt back up stunning / stunning in case of prolonged consciousness or recovery must be possible;
- if bleeding is not carried out in the device a quick release of the animal must be possible to guarantee a short stun-stick interval;
- a restraining device or method must suit the size and species and type of animals slaughtered;
- restraining must not cause negative impact on bleed-out, carcass or meat quality and should match the intended slaughter speed;
- good working safety must be achieved.

Animals enter a restraining device more easily, if there are no impediments like air draughts, sudden hissing or banging noises, dark areas, sparkling reflections, moving people or parts of the slaughter chain, slippery floor, inadequate floor incline or changes of structure or colour of walls or floor, and if the restraining device is well designed, e.g. shield the animal from distractions or does not appear too much a dead end. Consequently the stress and strain an animal experiences during restraint depends on quality of raceways towards the restraining device, construction of the restraining system itself, the degree of restraint (tightness or pressure), the time of restraint and individual experience e.g. during preslaughter handling or individual features of the animal (excitement, adverse reactions, weight, horns) (Grandin, 1998b; Grandin, 1996; Grandin, 1994b).

The restraining method should not cause defence movements or flight reactions of the animal, which can lead to incorrect procedures due to wrong positioning of the stunning or cutting instruments (Holleben, 2007). All restraining methods should use the concept of optimal pressure. The device must hold the animal firmly enough to facilitate slaughter without struggle but excessive pressure that would cause discomfort should be avoided. Struggling is often a sign of excessive pressure (Grandin, 2005).

Smaller animals can be lifted into the restraining device by hand, e.g. sheep and goats may be put by hand on a table or poultry are put in shackles or funnels. These animals may be also restrained by hand without the help of a sophisticated mechanical device. Heavier animals like cattle need more complicated technical equipment as well to hold them, e.g. if they break down, but also to ensure working safety (Holleben, 2007).

Knowledge and skills of the staff handling the animals and operating the devices is extremely important for reducing stress, strain and injuries during fixation and restraint and also for eliminating negative impacts on bleed-out, carcass and meat quality (Grandin, 1998a).

Concerning the different slaughter methods the restraining device has to hold the animals / restrict their movements but also allow further processing including:
- application of the cut and the holding during the bleeding period *(slaughter without stunning),*

- application of a stunning device and subsequent timely bleeding *(slaughter after stunning),*
- application of the cut and subsequent prompt stunning *(post cut stunning).*

3.1   Restraining for slaughter without stunning

Restraining for slaughter without stunning needs to make sure that the neck can be stretched, so that an optimum cut is possible. It is also important, that the throat wound stays open to enable fast bleeding and the loss of consciousness as quickly as possible. Mechanical and chemical stimuli (e.g. blood borne metabolites) on the wound have to be minimized as long as the animals have not yet lost consciousness (Grandin, 1993b). Adequate post cut restraint is vital for correct bleeding (Rosen, 2004).

3.1.1   Restraining of cattle for slaughter without stunning

Cattle can be restrained either in an upright position, rotated by 90 degrees lying on their side or rotated by 180 degrees lying on their back. Rotating is also practised to other angles than 90 or 180 degrees depending on practical and religious reasons. Restraining cattle by suspending their hind legs causes stress and pain and is not acceptable according to animal welfare standards (Gregory, 2005b) and European legislation.

Upright restraining is possible either in a box or a pen, often custom made, with the neck being stretched or lifted by means of a halter and lateral straps or chains. Calves can be restrained by hand in a semi closed box, their heads being stretched manually. More complicated technical equipment uses mechanical systems such as chin lifts as headholders. The most famous pen for upright restraint is the so called Cincinnati or ASPCA pen, consisting of a chin lift, a belly plate and a backpusher (Grandin, 1993b). This pen design has been constructed and modified by a number of slaughter house suppliers and has also been self built by slaughter plants engineering departments. Most of the rotating boxes can be operated in a similar manner to the upright systems, using a backpusher, a head restrainer and adjusting the sides of the pen. Another way to restrain cattle in an upright position is by using a double rail (center track) conveyor restrainer, in which the animals are placed with their legs straddling, not touching the ground, and their bodyweight being supported under the brisket and belly. When the animal reaches the front of the restrainer the head is stretched by a chin lift and then the cut is performed (Grandin, 1988). This kind of equipment is used also for calves and sheep, mainly in America where high slaughter speed is required.

Restraining cattle on their back is practised in rotating pens, in which the head is restrained, the body confined laterally and the animals turned on their backs. In this position the cut is performed, and afterwards the animals is rotated a little backwards to be released from the pen and shackled. One of the earliest types of a rotating pen was the so called "Weinberg" –pen. At this time the Weinberg pen provided a great advance towards better safety, compared to clamping the legs of an animal and pulling them down (Levinger, 1976). Since then other suppliers have adopted the principle (e.g. Facomia, France; Banss, Germany; Nawi, Netherlands) and also developed modified equipment with respect to practicability and animal welfare, for example new layout of head restraint or chin lift, neck yoke, pressure of side walls and head lift, mechanical control and smooth operation of turning, changing direction, angle and speed (Levinger, 1995). Rotating pens may turn the animal around it own axis or around an external axis (see also Dialrel WP2 report on spot visits).

Restraining cattle on their side is also possible using the same rotating pens used for turning them on their back, e.g. the Facomia pen. The rotating pens are then turned to 45 or 90 degrees or positions in between and the cut is performed while cattle are tilted. Smaller plants also use self built or modified equipment like claw trimming tables to which a headholder is attached to support the animals head after the cut, while cattle are in lateral recumbency.

Concerns about restraint have been expressed for all methods. Grandin (2005) proposed to evaluate the quality of a restraining device for religious slaughter of cattle under practical conditions by "percentage of animals rendered insensible within 10 to 15 seconds", "percentage that vocalize during handling and restraint", "percentage that are moved with an electric goad", "percentage that slip during handling" and "percentage that fall during handling". The author puts strong emphasis on factors that cause excitement because in her experience calm cattle collapsed more quickly and appeared to have a more rapid onset of unconsciousness and also a more relaxed animal will facilitate bleed-out (Grandin and Regenstein, 1994).

<u>Upright restraint</u> of cattle during slaughter without stunning was judged the better method even though rotating pens have been improved. However some upright systems have design flaws, which hinder good restraint, like excessive pressure on the animal, poorly designed headholder or chin lift or hyperextension of the head (Grandin, 2005; Grandin and Regenstein, 1994; Farm Animal Welfare Council, 1985). Regenstein and Grandin (1994) mention reactions of the animals due to irritation of the wound e.g. if the wound touches the metal parts of the neck frame. This provoked active movements and it may also slow down exsanguination. The author recommends on her homepage, reducing the pressure on the animals body immediately after the cut to achieve good bleed-out and ensure quick loss of consciousness. Berg (2007) also reported that construction and operation of an upright pen can contribute to pain, suffering and stress due to excessive pressure being applied by the back pusher or head holder or edges of the wound touching each other, the ground or metal parts of the pen. The author reported that hyperextension of the head contributed to insufficient cuts, because the operators were hesitant to touch the metal headholder with the knife (Berg, 2007). The Farm Animal Welfare Council visited plants using upright as well as turning pens and reported the difficulty of holding the animal after the cut so that the animal was fully supported as it collapsed and did not fall onto the wound when in the pen. It was concluded that combined the effects of the belly plate and the backpusher were essential to achieve this goal (Farm Animal Welfare Council, 1985).

Dialrel researchers have reported the following observations during examination of upright pens:

- Time between entering the pen and restraint was between 5 and 35 seconds. Afterwards between 2 to 6 seconds were needed in the quickest slaughterhouses before the cut was initiated. Sometimes it could take up to 5 minutes between beginning of head restraint and cut.
  Longer times to restraint does not always indicate deficient construction or handling as sometimes it was necessary for an operator to take that time. However longer times to restraint were often linked to impediments like slippery or irregular floor or hissing noises, excessive use of driving aids, inadequate headholder or neck frame and thus excited animals or a lot of effort needed to correct the position of the head in the headholder. Prolonged times between the beginning of restraint and cut were also due to lack of awareness of the operators or additional procedures like washing the neck. Whereas optimum construction can lead to the quick restraint of the head and performance of the cut within 5 seconds after the animal entered the pen.

- A lower frequency of vocalisation during restraint was noticed in an optimally constructed and operated pen for upright restraint. Whereas with an inadequate head holder and neck frame vocalisations were observed in 13% to 19% of the animals.

- Performance of the cut varied between operators (between 2 and 12 cuts performed), indicating that extensive skills are needed in an upright pen system. In extreme cases an average of 25 cuts were performed per animal.

- Physical reactions to the cut, like retraction or shivering movements could only be noticed if the neck was not hyperextended and not restrained too tightly.

- The management of the animals in the upright pens after the cut varied according to construction, e.g. with and without belly plates and operation routines. In some situations the animals were kept in tight fixation between the headholder and back pusher and with the neck kept stretched. In other pens the headholder and/or the back pusher were loosened, sometimes completely leaving the cattle to stand by themselves. In this example this could often prevent the wound surfaces of the cut, touching the metal parts of the neck frame. In other cases the wound surface touched the metal parts of the frame more frequently and lead to aversive reactions, like attempts to withdraw the neck. Animals were released from the upright pen between 26 and 173 seconds after the cut, longer time intervals did occur, if very large animals were trapped in the pen. In some situations the lower part of the opening in the front side of the pen could cause pressure on the lower neck during bleeding resulting in impaired blood flow and spraying of blood.

- Blood from the severed vessels spread over the wound, into the larynx and also entered the trachea. Stomach content could also spread over the wound, but only after the animals had been ejected from the pen.

Animals inverted on their backs for slaughter in rotating pens had a longer time interval from entering to full restraint, showed more vigorous and longer periods of struggling, increased number of vocalisations, more laboured breathing (especially in the inverted position), increased foaming at the mouth and greater serum cortisol concentrations and haematocrit compared to cattle slaughtered in an upright position (Koorts, 1991; Dunn, 1990). The Farm Animal Welfare Council (1985) called the rotation stressful and mentioned especially the gross discomfort due to weight and size of the rumen pressing upon the diaphragm and thoracic organs but also the unsatisfactory manner of operation. However it is not clear to what extent in these investigations the old rotary pen designs were used. The old rotary designs had suboptimal headholder systems and were possibly badly operated. In these systems in some situations cattle could escape from the restraint and often more than one attempt had to be made to rotate the animal into the inverted position (Koorts, 1991). Tagawa et al. (1994) rotated healthy Holstein cows into right lateral and dorsal recumbency without slaughtering them, using an operating table, to which the cows were strapped. They concluded that lateral recumbency and to a greater extent the supine position (on the back) exerted a strong stress which affected respiratory function. The plasma cortisol concentration increased with change of position. Values increased to more than three times the control values, however this only was after 30 minutes of being in the supine position. The arterial oxygen tension and oxygen saturation were significantly decreased directly following changes in body position and the decrease was most pronounced when cattle were restrained in a supine position (Tagawa et al., 1994). Decreased arterial oxygen tension following changes of position to right and left lateral and dorsal recumbency was also found by Wagner et al. (1990), while no significant changes occurred in heart and respiratory rate as well as other blood gas values. Van Oers (1987, see Gregory (2005b)) found more vigorous struggling when head restraint was applied after an animal had been inverted in comparison with head restraint before inversion. This was also observed during the Dialrel WP2 plant visits, as also the following:

- The time interval between entrance into the pen and restraint of the head (if head restraint was performed), was between 13 and 100 seconds. Longer time intervals often indicated difficulties in restraint due to inadequate construction of the headholder. In some pens a halter or a chain was used additionally to achieve

sufficient restraint of the head and neck. If the head of the animal was not well restrained before turning, more struggling was noticed and sometimes the neck was distorted during the restraining procedure, this was because the operators tried to catch the head of the animals during or after turning.

- Turning was performed over the right or left side of the animals within 8 to 15 seconds. When the animals were turned over an external axis, turning times were longer (average 52 seconds). The cut was performed between 10 and 60 seconds after the beginning of turning. Reactions during turning were wide open eyes, short and continuous bouts of struggling (often repeated over several seconds), attempts to raise the head and body, vocalization (up to 15% of cattle) and laboured pressed respiration especially in the upside down position. It is worth noticing, that in this context reactions of cattle are difficult to record in a predominantly enclosed pen design and they can be masked by the restraining system (Grandin and Regenstein, 1994).

- When the head was well-held by the headholder, the cut was started in 2 to 4 seconds after the end of turning, whereas if the position of the head had to be corrected after the animal was turned it could take up to one minute and sometimes even longer. Performance of the cut varied between operators (between 1 and 13 cuts were performed in the plants assessed during WP2).

- Movement reactions to the cut such as withdrawal or shivering movements could be noticed if the neck was not too tightly restrained. Often after the cut, the headholder was loosened to improve bleeding. This enabled some movement of the neck, which could be vigorous and also allowed the wound to make contact with metal parts of the headholder in these animals.

- After the cut the animal was either left in the inverted position for up to one minute (in individual cases longer), or it was turned back to the upright and sometimes ejected only a few seconds after the cut.

- Blood and rumen content often spread over the proximal and distal wound surfaces and also entered the larynx and trachea, while the animals were lying on their back. This depended on the degree of extension of the neck and the position of the cut.

Aspiration of blood and refluxing gut content after the incision was considered a welfare concern after slaughter without stunning. Though this problem was mainly associated with the inverted position, it occurred with the upright position for both Halal and Shechita slaughter (Gregory et al., 2009). The authors examined bovine respiratory tracts following Shechita and Halal slaughter without stunning, and also in captive bolt stunned animals. During all the treatments animals received the cut in the upright position. The study found blood lining the inner aspect of the trachea in 19% of the Shechita, 58% of the Halal and 21% of the stunned/cut cattle. Blood was found in the upper bronchi of 36% of Shechita, 69% of Halal and 31% of stunned/cut cattle. Ten percent of the Shechita, 19% of the Halal and 0% of the stunned/cut cattle had fine bright red blood-tinged foam in the trachea. Blood covering the larynx was recorded for all cattle. It was concluded that concerns about suffering from airway irritation by blood could apply in animals that are either not stunned before slaughter or do not lose consciousness rapidly whilst blood is present in the respiratory tract (Gregory et al., 2009). These concerns are based on the fact that fluid in the respiratory tract in conscious animals leads to irritation of sensory receptors lining the airway, and in particular the receptors on the glottis and the carina of the trachea. In animals with intact vagus nerves there could have been a cough or expulsion reflex, but coughing would be absent when the vagi had been severed (Canning, 2007) though lower airway irritation may still occur through sympathetic-spinal afferent pathways (Quin et al., 2007). Additionally blood impacting the

glottis could cause upper respiratory tract irritation, which under normal circumstances activates the cranial (superior) laryngeal nerve which would not be severed by the cut (Gregory et al., 2009).

Restraining of cattle in lateral recumbency is another practice used to restrain animals during slaughter without stunning. Petty et al. (1994) investigated Shechita under commercial conditions and conventional slaughter after captive bolt stunning in cattle restrained either in an upright position or turned to 90 degrees. They concluded that in lateral recumbency cattle were less stressed compared with lying on their backs, as the rumen did not press the diaphragm and therefore did not cause breathing difficulties. Nevertheless some pressure on the internal organs would still be present even in lateral restraint (Tagawa et al., 1994; Petty et al., 1991). Labooij and Kijlstra (2008) analysed the current knowledge for rotating restraint, especially with regard to the situation in the Netherlands, and recommended that the current equipment for turning should be devised and developed to improve restraint while allowing partial sideways rotation for easier performance of the neck cut. Experiences during the Dialrel project revealed, that lateral recumbency can help to avoid some problems like pressure on the aorta, major veins and diaphragm. Turning in lateral recumbency systems is usually shorter and the animals can be supported during and after breakdown with less pressure being applied. However other difficulties may arise as the performance of the cut has to be adapted to this position. Construction and operator deficiencies can also lead to problems e.g. with the post cut wound management similar to the inverted position. According to experience in the plants which were assessed during WP2 by the Dialrel veterinarians, the time between start of head restraint and cut varied from between one minute to more than 6 minutes. Turning to 90 degree took between 8 and 13 seconds. The number of cuts performed ranged from 4 to 13. Retraction movements could be noticed in response to the cut. Cattle were released between 112 and 193 seconds after the cut. In systems which turned the animals to 45 degrees, turning times were shorter. Both, turning and cutting were performed usually within 10 seconds after entrance into the pen.

From the literature and experiences within the project it was not clear whether turning over on the right or the left side was preferable. This might have an influence on pressure applied to the rumen, the forces pulling on the trachea and pressure on the vessels leading into the wound thus influencing blood flow.

In Turkey it was found during WP2 spot visits that some abattoirs employ methods that shackle the free standing cattle by one leg. The animals were hoisted until only one shoulder and the head supported the weight of the animals. Sometimes the animal was fully hoisted up first and then lowered onto its head and shoulder. Afterwards the neck cut was performed, before hoisting was completed. Upright pens were also used to hold the animal, and then one of the hindlegs was shackled through the gap underneath before opening the gate. Afterwards, the animal was dragged out and half hoisted for neck cutting. This method was used for both Halal and Shechita. The average period from exit to exsanguination was 67 seconds in that case. During hoisting cattle often vocalized, struggled and attempted to regain posture.

With all types of restraint in cattle it is possible, that stress before the cut and the position of the animal during and after the cut can have a marked impact on bleeding and bleed-out (see 2.8.3).

3.1.2 Restraining of sheep and goats for slaughter without stunning

Sheep and goats can be restrained either in an upright position, lying on their side or lying on their back (Levinger, 1995). Rotating is also used at angles other than 90 or 180 degrees. Restraining animals, even small animals like lambs or kids, by suspending their hindlegs is

not according to animal welfare standards. Nevertheless it is still performed in Europe (see Dialrel WP2).

Sheep and goats are restrained upright, mostly by hand or the operator taking the animal between his legs, limiting backward movement by use of a fence or wall and stretching the neck of the animal by hand. Upright restraint can also be performed in specially constructed restrainers like the one of the US Northeast sheep and goat marketing program, which was developed for single animal slaughter on farms (Regenstein, 2000). For these systems the cut and also post cut handling has to be carefully coordinated and it is advisable that there is at least a one-minute interval before further procedures are started. For higher slaughter speeds centre track double rail conveyor restrainers have been constructed in a similar way to those used for cattle (Levinger, 1995; Giger et al., 1977).

Studies revealed that there was greater difficulty in moving sheep through a raceway for a second time if they had been held in dorsal recumbency for 30 seconds on the previous occasion (Rushen, 1986; Hutson, 1982; Hutson and Butler, 1978). It is important that sheep are restrained promptly and without hesitation and with as little pressure as possible to avoid unnecessary forces (Ewbank, 1968). Holding or lifting them by grasping their wool should not be done (Holleben, 2007).

According to Dialrel experience, restraining sheep and goats on their side is performed by lifting them onto a table or laying them on the floor, where they are held by hand or their legs may be attached to the table by straps or chains. The head of the animal during and after the cut is either handheld or supported by a table or grating. V-shaped restrainers are used to process the animals towards the table, out of which the animals are lifted by hand. For higher speeds mechanical devices holding the animals between adjustable side walls are sometimes used to turn the animal on their side and into the required direction according to religious requirements. Again animals are put into these restrainers by hand and the head is supported by hand during and after the cut. Sheep have been observed shackled by one leg (Catanese et al., 2009; Cenci Goga et al., 2009) before sticking for Kosher slaughter (up to 17 kg liveweight, line speed exceeded 200 sheep/ h) and for Halal slaughter (sheep up to 55 kg liveweight, line speed could exceed 150 sheep/ h). Shackling time averages ranged between 1 and 4 minutes before performance of the cut, but can reach 5 minutes if the time was needed to sharpen the knife or if the operator had to approach first to perform the cut. During shackling before sticking some sheep hung calmly whereas others struggled. Struggling was increased if a sheep touched another struggling sheep. Sheep reactions also included turning the head to the side and apparently looking around, kicking with the hind leg and vigorous struggling including movements of the whole body. In the last case, the movements were thought to be escape behaviour. Struggling and vocalisation sometimes occurred in response to the cut.

3.1.3   Restraining of poultry for slaughter without stunning

During Shechita slaughter, Barnett et al. (2007) described that each chicken is restrained manually by a person holding both its legs in a raised hand and supporting its back, with its wings folded, on the opposite forearm and other hand. The shochet is then able to extend the bird's head in his left hand with his thumb against the ventral surface of the bird's upper neck close to the beak and cut all the blood vessels with the knife in his right hand. The bird is then passed to a third person who places it into a bleeding cone (Barnett et al., 2007). Other methods are to place the bird in a cone before performing the cut or placing the birds in lateral recumbency for Halal slaughter. According to Dialrel experience, restraining poultry for Shechita was performed manually or by shackling chickens and turkeys before cutting, although the latter was not according to kosher rules. During shackling chickens could hang calmly or show wing flapping which in some cases was vigorous and long lasting.

3.2 Restraining for stunning prior to neck cutting

With electrical and mechanical stunning methods it is important to place the stunning device accurately on the head. This usually requires individual restraint of the animals. An overview on restraining methods is given in table 3. Bleeding is performed either in the restraining device or on the shackled animal after it has been released from the restrainer.

Table 3: Overview on restraining methods for stunning of individual animals

	Cattle	Sheep	Poultry
Electrical stunning	Single animal pen with manual electrode placement	Single animal restrainer or confinement or v-shaped restrainer / centre track conveyor with manual electrode placement	shackle
	Single animal pen with automatic electrode placement, e.g. Jarvis, Banss	Manual restraint on a table in recumbent position	cone
	Halter handheld	Fixation between operator's legs/ near to a wall or by hand	by hand/ sitting in a crush
		Shackling (lambs)	
		Stunning in a group	
Mechanical stunning	Single animal pen, manual placement of stunning device	Single animal restrainer or pen with manual placement of the stunner	shackle
	Halter, handheld	Fixation between operator's legs/ near to a wall, chin handheld	Cone
			by hand/ between operator's legs (turkeys)

If the ten general requirements listed at the start of Section 3 are not achieved, inadequate restraint can lead to incomplete stunning by misplacement or interrupted application of the stunning device such as tongs and captive bolt gun. It can also lead to late bleeding if the animals are not transferred sufficiently quickly to the bleeding position (Adams and Sheridan, 2008; EFSA, 2004; Holleben et al., 2002; Ilgert, 1985).

In cattle concave shaped tables for the head in combination with a back pusher improve targeting bolt position in cases of high slaughter speeds (Holleben, 2007). However too tight fixation of the head (e.g. by a chin lift and neck yoke in a poorly designed system) will lead to increased stress and prolonged times until head restraint (Ewbank, 1992). In Germany (Troeger, 2002) and New Zealand (Gilbert, 1993) systems with automatic electrode placement by neck yoke and nose electrodes have been developed for electrical stunning of cattle. These systems are used for conventional and for Halal slaughter. In Europe, manual placement of electrodes in traps is practised in small scale slaughter facilities (Wenzlawowicz, 2010). During the Dialrel W2 spot visits electrical stunning was also performed in a rotary pen, after the cattle had been turned on their backs within 13 seconds. This procedure then was changed, so that turning was performed more quickly and electrical stunning was applied during the turning process. The Halal cut was applied on the turned animal whilst lying on the back. The animals then stay in the pen for 3 to 4 minutes during bleeding before being partly turned upside down and then released from the pen for shackling.

Sheep naturally follow each other and will often line up and freely enter a well shaped restrainer or trap, usually showing little or no agitation . However, incompetent handling, such as grabbing of fleece or putting pressure on wrong parts of the body during manual restraint will lead to increased stress and arousal (Hutson, 1993). When sheep are group stunned in a pen, they may hide their heads under animals which makes it difficult to correctly place the electrodes. Also other sheep in the group may make physical contact with the animal that is

being electrically stunned possibly resulting in painful electrical shocks. Sheep should be individually restrained manually in a trap or in a restrainer, to minimise incomplete stunning and painful electric shock from group mates (EFSA, 2004, p.77).

In poultry most concerns have been expressed on the practice of live bird shackling. The pressure applied during shackling increases with deformation of legs or increasing weight and size especially in turkeys. Nevertheless, some modern shackle lines are designed to accommodate birds of different sizes but these are not commonly used under the existing processing conditions (Gentle and Tilston, 2000; Gregory, 1998b; Gregory and Wilkins, 1990; Gregory et al., 1989). Shackling time has been limited in the respective European slaughter legislation and even the phasing out of live birds shackling is being discussed. In gas stunning systems poultry may either stay in their transport crates or they may be tipped automatically from the crates onto a belt conveyor. Consequently there is no need for individual restraint in these systems. Dump module systems used for tipping the birds out of the transport crates must be constructed in a way to achieve the birds sliding – not falling - out of the crates onto a sufficient large area of the belts. This is necessary to minimize the higher frequency of red wingtips from wing flapping, associated with some of these systems.

3.3 Restraining for post neck cut stunning

Generally restraint for post cut stunning involves the same difficulties as slaughter without stunning until the stunning procedure is performed. Firstly restraint has to make sure that the neck can be stretched in order to perform an optimum cut. Secondly the throat wound has to stay open to enable fast bleeding. Additionally to these requirements a post cut system must allow secure positioning of stunning devices immediately after the cut. The length of the interval between cutting the animal's throat and applying the stunning equipment depends in particular on the way in which the animal is restrained (Binder, 2010). This time period for cattle may either depend on technical premises concerning the construction of the restraint device (e.g. if the head of the animals cannot be accessed using the stunning devices) or a prolonged time interval may be due to improper performance (e.g. the person operating the stunning equipment being not ready to apply the effective stunner immediately). Also religious reasons may contribute to delayed stunning after the cut (Berg, 2007).

Eight calves were observed by the first author in one slaughter plant to be restrained by hand in a semi closed box, their heads stretched manually. The animals were post cut stunned by captive bolt on average 3 seconds after the cut (range 1.8 to 4.6 seconds). If restrained upright, the animal is in a standing position when both its throat is cut and when the stunning device is applied. In spite of the stun being possible within 5 seconds after the cut when it is applied while the head is being held by the neck frame, Berg (2007) measured the time intervals from starting the cut to the post cut stun of cattle in an upright pen as being between 30 and 40 seconds and sometimes even longer (60 to 120 seconds). For post cut stunning of cattle in a rotary pen design, stunning should be performed immediately after the cut (Gsandtner, 2005), however between 12 and 15 seconds were measured between the cut and application of stunning. This time interval was needed to rotate the animal back from the cutting position into a position where the captive bolt apparatus could be placed (Binder, 2010; Berg, 2007). In one plant assessed by Dialrel, there was 26 seconds between cutting and stunning when cattle were turned on their back for the cut. When turning to 45 degrees, non penetrative captive bolt stunning could be performed very soon after the cut.

In conclusion from the point of view of animal welfare it is vital, that restraint for post neck cut stunning allows both optimum cutting position and the application of a stunning device immediately after the cut.

To summarize, requirements for restraining as well as possible welfare relevance of improper restraint depend on slaughter method and animal species. For all slaughter methods it is difficult to restrain animals of different sizes and shapes. This applies in particular to huge animals like adult cattle. The special challenge concerning slaughter without stunning is to manage the restraint during and after the cut. Concerns are summarized in chapter 4.

4   Slaughter methods (Principles and concerns)

4.1   Neck cutting without stunning

Slaughter without stunning is performed where religious rules do not allow stunning. According to the Dialrel glossary, religious slaughter means slaughter according to religious rules which does not necessarily mean that slaughter is carried out without stunning. Issues of restraint have been already mentioned in chapter 3. In the following the question of pain during the cut and time to loss of brain responsiveness after the cut will be discussed.

4.1.1   The cut

The question whether the cut is painful, even if it is performed by a perfectly trained operator with a perfectly sharp knife on a calm animal is most important with regard to animal welfare during slaughter without stunning. Pain in general, perception of pain and different qualities of pain have been described in chapter 2.1. During slaughter nociceptive pain produced by mechanical forces of cutting cannot be influenced by a clean cut. Meanwhile the severity of inflammatory pain, produced by tissue damage can be mitigated but not eliminated by a good throat cut (Brooks and Tracey, 2005; Woolf, 2004). Whilst wounds which involve tearing of tissue or multiple cuts will invoke greater nociceptor activation than clean cuts, nociceptors will still be activated even in response to a single neck cut or deep cut to sever the blood vessels of the neck, irrespective of the sharpness of the knife. Based on physiology it is known that large wounds usually elicit major pain responses (EFSA, 2004, page 21).

Grandin and Regenstein (1994) described little or no reaction to the throat cut by calves and cattle, restrained in low-stress upright restraint system, except for a slight flinch where the blade first touched the throat. The animals made no attempt to pull away and there were almost no visible reactions of the animal's body or legs during the throat cut. Little or no reaction to the cut occurred in 6 calves reported by Bager et al. (1992).

Other scientists argue that pain will be substantially involved. They refer to a cut in order to achieve rapid bleeding will cause substantial tissue damage in areas well supplied with nociceptors (Kavaliers, 1989). Any cut intended to kill the animal by rapid bleeding will greatly activate the protective nociceptive system for perceiving tissue damage and cause the animal to experience a sensation of pain (EFSA, 2004, page 21). The tissues that are cut include skin, long hyoid bone muscle, trachea, oesophagus, both jugular veins, both common carotid arteries, both trunci vagosympathici, both nervi recurrentes, both trunci jugulars and parts of the long throat muscle (König, 1999). Lamboij and Kijlstra (2008) in their review support the above mentioned view that the neck cut itself will cause the sensation of pain since this area of incision has a high density of pain receptors. In some animals however a temporary acute shock may block the sensation or expression of pain (Lambooij and Kijlstra, 2009).

Reports on behavioural reactions of animals during slaughter without stunning are often based on anecdotal observations. Where the conditions of the cut are not clearly depicted (e.g. sharpness of the knife, skills of the operator), or where it is not mentioned whether the reactions to the first cut or to a multiple cut or back up cut are described. An additional

difficulty in interpreting reactions to the cut is that animals may not be able to react or reactions are masked due to the animals position (e.g. shackled or within a head restrainer), due to natural freezing behaviour or due to limitations in the reactions because the necessary tissues have been severed (e.g. vocalisation not being possible through a cut trachea (see above)). Fainting during haemorrhagic shock may also make movement difficult. Hence low levels of behavioural response following throat-cutting do not necessarily indicate that the individual was not experiencing pain (EFSA, 2004, page 24; Schatzmann, 2001). Alternatively it is stated that the low behavioural responses to the cut demonstrates that the cut is not painful (Levinger, 1995; Levinger, 1976)..

Nevertheless the most important tool for assessing pain and suffering, especially in field conditions, is observing the behaviour of the animal. Additional information may be obtained from basic physiological measurements, such as heart rate, respiration rate and body temperature (Barnett, 1997). These parameters take time to react or may be influenced by involuntary reflex reactions to the cut like loss of blood volume. While pain induced distress might normally be assessed using adrenal cortex responses, these cannot be accessed in neck cut animals, because ACTH is prevented from reaching the adrenal glands via the blood. Furthermore glucocorticoid responses take more than 2 minutes to be evident. Hence the lack of an increase in blood cortisol reported in some studies (Tume and Shaw, 1992) is not surprising.

Barnett et al. (2007) investigated kosher slaughter, where each bird was restrained manually and its neck presented to the specialist slaughterman. The results showed that 4 of 100 birds responded physically to neck cutting. The birds showed a mild response. This meant a minor local movement of neck or head without body and/or leg movements.

Klein (1927) observed reactions of a sheep after slaughter without stunning in upright position and concluded from the immediate flight that the cut had been painful. A young castrated bull after having been released from being tied down for the cut, showed defence movements, got up and fled following the cut. This was also interpreted as a reaction to pain (Klein, 1927). Hazem et al. (1977) reported heavy defence movements after the cut in 1 out of 10 calves, slaughtered by Shechita, which hindered the EEG measurements. Apparently this animal was very nervous and already reacted vigorously to noises and handling before the cut.

Especially for Shechita it is stated that the exquisite sharpness of the knife (Chalaf), coupled with the smoothness of the incision means that there is minimal stimulation of the incised edges, typically below a level adequate to activation of pain pathways. This can be compared to the experience of surgeons, who have cut themselves in the course of an operation and only noticed it well after the event (Rosen, 2004). It must be taken into account however that the throat cut involves a major tissue damage over a large area and that pain is not exclusively related to the quality of the cut, see chapter 2.1. With regard to humans when injuries were deep (e. g. fractures, crushes, amputations and deep stabs), 72% of subjects experienced prompt pain and 38% perceived pain only later. When injuries were limited to skin (e.g. lacerations, cuts, abrasions, burns), 53% of subjects had a pain free period immediately following the injury. In the case of deep injuries (fractures) where there was no immediate pain, there was instead an initial feeling of numbness at the wound and persistent pain developed later when the pressures associated with haemorrhage, oedema and inflammation developed, and when pain receptor agonists released from the injured tissue accumulated at the wound (Gregory, 2004; Melzack et al., 1982).

The average number of cuts as reported by Gregory et al. (2008) was 3.2 cuts during Shechita and 5.2 cuts during Halal slaughter of cattle. In sheep, according to the experience of the Dialrel WP2 members, the minimum number of cuts required to sever the major blood vessels of the neck for Halal (without stunning) and kosher slaughter ranged from 1 to 6. For cattle

either one or up to 60 sweeps of knife have been found. For poultry usually one cut was performed. Additionally to multiple cuts, after withdrawing the knife from the wound, additional cuts were sometimes performed during Kosher and Halal slaughter. Each time the knife touches the surface of the wound the potential exists for further nociceptor activation. Even if the knife blade is twice as long as the width of the throat, there are limitations to cut the neck of large cattle with a small number of cuts. This is due to the fact that according to the area to be cut the length of the blade increases disproportionately depending on the pressure that can be applied by the operator (Adams and Sheridan, 2008).

Especially in sheep but also in cattle an additional aspect may be thick wool or coat, which may have to be parted before the cut. Otherwise this would constrain the blade during cutting and could cause blunting of the blade. Blunt blades are especially welfare relevant if the neck is not sufficiently stretched to fixate the flexible skin around the neck of sheep or cattle (Wenzlawowicz and Holleben, 2007).

Measurement of the electrical activity of the brain to assess noxious stimuli has been described in chapter 2.6.2. Recent advancements in electrophysiology have allowed quantitative analysis of the electroencephalogram (EEG) in response to painful/noxious sensory input, allowing the experience of pain to be more precisely assessed in humans and animals. This methodology has been applied to the question of pain during slaughter of calves by ventral-neck incision. In a series of experiments the results showed clear evidence for the first time that the act of slaughter by ventral-neck incision is associated with noxious stimulation that would be expected to be perceived as painful in the period between the incision and loss of consciousness (Mellor et al., 2009). First the use of changes in the EEG power spectral and a minimal anaesthesia model was validated for the assessment of noxious sensory input using amputation dehorning as a noxous stimulus (Gibson et al., 2007). Then the model was used to investigate the impact of ventral-neck incision without prior stunning (Gibson et al., 2009b). The results demonstrated that ventral neck incision produced changes in the EEG indicating that it was a noxious stimulus and therefore could be perceived as painful in conscious animals. This was then confirmed in the second study addressing the question whether the EEG responses after ventral neck incision were due primarily to the cutting of neck tissues or to interruption of blood flow to and from the brain. The results demonstrated that the predominant noxious stimulus was cause by the transection of neck tissue not the loss of blood flow to and from the brain (Gibson et al., 2009a). In sheep there is no direct EEG data that demonstrates pain in response to the cut, however based on the physiological similarities between sheep and cattle, it stands to reason that the neck cut in non stunned sheep will cause pain (Hemsworth et al., 2009).

Nerves severed during the neck cut have been described by Gregory (2004, page 96) to be able to proceed signals for up to 4 seconds. Direct activation of neurones during transection of the nerve results in an intense but brief injury discharge in the afferent nerves. The overall effect is likely to be a sense of shock, comparable to an electric shock. Subsequently, undamaged nerve endings and also nociceptors in the neck wound respond if stimulated or disturbed especially from cold drafts and mechanical effects depending on the way the wound is managed before consciousness will be lost (Gregory, 2005b; Gregory, 2004). Nerve conduction velocities ensure that activation of brain centres following major cutting injury occurs within milliseconds. Therefore, the potential experience of pain is directly relevant to the events following the neck cut (Hemsworth et al., 2009).

During the Dialrel spot visits even under apparently optimum conditions, reactions to the cut as vocalising or exhaling (as long as the trachea was intact), retracting movements, struggling or shivering have been found especially in cattle during Halal and Shechita slaughter without stunning in both turning pens and upright systems. Reactions in sheep have been observed as

struggling directly after the cut but also shivering. Reactions of poultry have been retraction movements and wing flapping.

In conclusion it can be stated with the utmost probability that animals feel pain during the throat cut without prior stunning. Whereas the actual cut itself can only be evaluated using behavioural signs, questions remain about standardisation of cutting techniques. Because in all probability animals are able to experience pain during and after the cut, the question of duration of consciousness is very important. This applies as well for a smooth cut performed by a skilled operator. Risk factors for increased pain include increased number of changes of direction of the cut, increased number of cuts, wound manipulation (e.g. second cut), insufficient length of the blade, increased cutting time, a blunt blade, nicks on the blade, increased diameter of the neck, increased flexibility of the skin due to insufficient tension of the neck tissue during the cut, thick wool or coat and excitable animals.

4.1.2 Time to loss of consciousness

After the blood vessels are cut, as a consequence of blood loss, there will be deficiencies of nutrients and oxygen in the brain and other organs and consciousness will be lost. Further blood loss will disrupt brain function irreversibly and result in death. It is also possible that animals regain consciousness during bleeding (see chapter 2.8 and also below). The duration of overall consciousness is of particular importance. Its duration depends on the method of restraint, the quality of the cut as well as the animal species (see also chapters 2.7 and 2.8).

Table 4: Time to loss of brain function in cattle (means and/ or ranges (s))

Type and number of animals (age, weight)	Time post cut to indicators for loss of consciousness	Parameter for loss of consciousness, used in the respective study	Source
8 calves (1 week old)	34s (1 animal), 65-85s (others) 123-323s 132-326s	EEG amplitudes not consistent with sensibility Periodic resurgence of possible sensibility Isoelectric EEG	(Newhook and Blackmore, 1982a)
10 calves (40-60 kg)	10 s (up to 18s, 24s)[1] 23 s	Relevant EEG changes[1] Isoelectric EEG	(Schulze et al., 1978 and original report)[1]
8 calves (30-40 kg)	17 s (12-23) 23 s (14-28)	Loss of VEPs [2] Flat ECoG	(Gregory and Wotton, 1984c)
1 calf (35-55 kg), 6 weeks old	79s	EEG amplitudes not consistent with sensibility	(Devine et al., 1986a)
6 calves (4-8 weeks old)	10s	ECoG analysis (power content and frequency)	(Bager et al., 1992)
4 cattle (170 kg), Shechita	10.8s (8.7-12.8)	ECoG isoelectric	(Kallweit et al., 1989; Daly et al., 1988)
8 cows (436 kg) Shechita	7.5s (5-13) 28s (9-85) 72 s (19-113) 77 s (32-126) 55 s (20-102)	Start of HALF[2] Duration of HALF ECoG <10µV Loss of SEPs [2] Loss of VEPs [2]	
2 calves (7 day old) (severe exteriorised vessels) 1 calf (7 day old) 1 bull 13 month old	16-40/ 30-47 5/41 3 (fractured leg)/ 20	Loss of ability to stand /Loss of coordinated attempts to rise (only animals with satisfactory cut and no occlusion)	(Blackmore, 1984)
174 adult cattle	19.5 s (maximum 265s)	Time to collapse	(Gregory et al., 2010)

[1] the original report and data of the project (Hazem et al., 1977) revealed that, though the authors concluded loss of consciousness being highly probable in calves after 10 seconds, they recorded unchanged EEG until 18 seconds after the cut and in one animal, which had to be re-cut because of obviously slow bleeding, the EEG showed only very small changes until 24 seconds after the first cut

[2] HALF = high amplitude low frequency waves; VEPs' = Visual evoked potentials, SEPs' = somatosensory evoked potentials

The precise time after the cut at which non-stunned animals become insensible to pain remains a major methodological challenge, and although attempts have been made using changes in different features of brain electrical activity and behavioural changes, there are limitations in how the findings can be interpreted (Hemsworth et al., 2009; Tidswell et al., 1987). Nevertheless from the table 4 it can be concluded that most of the cattle seem to lose consciousness between 5 and 90 seconds after the cut, but even under laboratory conditions possible resurgence of consciousness has been assumed for more than 5 minutes. For Daly et al. (1988) the most striking feature of these results was the extent of the variations between individual animals in the duration of brain function. Regarding the small number of animals investigated, it becomes evident that only under field conditions the percentage of animals with prolonged consciousness can be estimated at all. Therefore the investigation of Gregory et al. (2010) was mentioned for comparison, a study which was conducted in a plant performing Halal slaughter with highly skilled staff. Fourteen percent of the cattle stood up again after the first collapse, before finally collapsing. The average time to final collapse for all the cattle was 20 seconds. Between the first and the final collapse it is likely that they periodically resumed consciousness. Eight percent of the animals took 60 seconds or more to achieve their final collapse, only one of which had incompletely severed carotid arteries (Gregory et al., 2010). These results show clearly that there is a marked difference between investigations under laboratory conditions and field studies.

Table 5: Time to loss of brain function in sheep (means and/ or ranges (s))

Type and number of animals (age, weight)	Time post cut to indicators for loss of consciousness	Parameter for loss of consciousness , used in the respective study	Source
17 sheep (16-30 kg)	4-6s (8s) [1] 13s	Relevant EEG changes[1] Isoelectric EEG	(Schulze et al., 1978 and original report)[1]
5 lambs (1 week old) and 16 adult sheep	2-7s 10-43s	EEG amplitudes not consistent with sensibility Isoelectric EEG	(Newhook and Blackmore, 1982b)
1 lamb (3 month old) 1 lamb (3 month old) decapitated	7s 48s 8s 20s	EEG <10µV Isoelectric EEG EEG <10µV Isoelectric EEG	(Tidswell et al., 1987)
10 sheep (30 kg)	8-22s	EEG amplitudes not consistent with sensibility	(Devine et al., 1986a)
20 mature ewes	14 s (mean) 95% within 22 s	Loss of VEPs[2]	(Gregory and Wotton, 1984a)
4 sheep Halal (48 kg) 9 shp. Shechita (30 kg)	12.5s (5.4-19.6) 12.4s (7.7-17.2)	ECoG isoelectric	(Kallweit et al., 1989)
1 sheep, 4 times clamped both carotids	6-8s	Relevant EEG changes (large waves of low frequencies)	(Levinger, 1976; Levinger, 1961)
Unknown number and age	3.5-5s 12-15s	Duration of normal EEG Relevant EEG changes	(Nangeroni and Kennet, 1963)
3 lambs (7 day old) 2 adult sheep (exteriorised vessels severed)	2-3s / 9-10s 3-4s / 8-9s	Loss of ability to stand /Loss of coordinated attempts to rise (only animals with satisfactory cut and no vessel occlusion)	(Blackmore, 1984)
1 sheep exteriorised carotids severed	10s	Loss of ability to stand	(Levinger, 1961)

[1] the original report and data of the project (Hazem et al., 1977) revealed that, though the authors concluded loss of consciousness being highly probable after 4 to 6 seconds in the publication, they recorded unchanged EEG until 8 seconds after the cut and concluded in the original report that sheep lost consciousness latest after 10 seconds.

[2] VEPs' = Visual evoked potentials, SEPs' = somatosensory evoked potentials

Following the results summarized in table 5, sheep seem to lose consciousness within 2 to 20 seconds after ventral neck cut. Loss of consciousness seems to be quicker than in cattle and with less individual variation. However with regard to the small number of experimental animals in relation to the aforementioned large variety of possible impacts on bleeding, exceptionally long periods of consciousness may not be excluded. They are probably less frequent than in cattle. During Dialrel spot visits, even in a plant with highly skilled staff, 2 mature sheep out of 400 showed signs of regaining consciousness during bleeding after nearly 2 minutes. But in only one of them bleeding was noticed to be slower than normal.

In poultry Barnett et al. (2007) concluded, after studying loss of ability to retain a standing posture in 41 Kosher slaughtered meat chickens (1.7 to 3.3 kg liveweight), that they lost consciousness on average between 12 and 15 seconds after the cut. One bird remained conscious for up to 26 seconds (Barnett et al., 2007). This corresponds to EFSA (2004, page 117-118), where from the available scientific literature it was concluded that a minimum of 25 seconds bleed-out time will be necessary to achieve brain ischemia through blood loss and avoid return of consciousness. Gregory and Wilkins (1989a) clearly demonstrated that cutting all the major blood vessels in the necks of electrically stunned chickens resulted in loss of blood amounting to more than 2 percent of body weight in less than 25 seconds after neck cutting. Although direct experimental scientific evidence is lacking, it can be speculated that this amount of blood loss in poultry may induce brain ischemia following slaughter and, hence, prevent return of consciousness (EFSA, 2004, page 117).

Delayed loss of consciousness can be due to manifold impacts (see chapter 2.8.3.) and severing both common carotid arteries is probably the most important requirement to achieve good bleeding during religious slaughter. Studies on frequency of failure to cut both common carotid arteries during religious slaughter are rare. Gregory and Wotton (1986) dissected the necks of chickens after manual religious slaughter and found both common carotid arteries were severed for 58 percent of chicken slaughtered by Shechita and 100 percent of chicken slaughtered Halal. In cattle Gregory et al. (2008) after investigation of the severed vessels during Shechita and Halal slaughter without stunning reported 21 out of 231 animals (9%) slaughtered by Shechita where a carotid artery was incompletely cut or not cut at all. Four of these animals had inadequate cutting in both arteries. The prevalence of failure to cut a carotid artery was higher during Shechita than Halal slaughter (6% and 1%, respectively).

In summary part of the welfare concerns about performing a ventral neck cut on an unstunned animal arises, because following the cut it may take some time to achieve unconsciousness. One of the main concerns about slaughter without stunning is, that animals will perceive pain or will be further processed and exposed to painful stimuli, e.g. released from restraint or shackled during the period they are still conscious.

4.1.3   Clinical signs during the post cut period

The clinical appearance of an animal in the period following the throat cut without stunning depends on characteristics of the animal (species and breed), state of arousal, the method of restraint, the quality of the cut (tissues severed, bleed-out), and it must be interpreted in relation to the time after the cut. Some features are given here especially with regard to what can be expected and what might indicate remaining or regaining consciousness (see also chapter 2.6).

As a compensatory response to a hemorrhagic shock caused by bleeding, heart rate will increase, also local vasoconstriction of arterioles and muscular arteries will occur, shifting of extravascular and venous reserve fluids to the circulating blood volume will take place, all these will contribute to more or less maintenance of perfusion pressure in vital organs up to a certain moment (Guiterrez et al., 2008). Schulze et al. (1978) report that after the Shechita cut,

heart rate increased within 40 seconds to 240 beats per minute for calves and to 280 beats per minute for sheep. In halothane-anaesthetised calves tachycardia developed from 140 seconds after ventral neck incision onwards (Gibson et al., 2009b).

Physiological reactions to blood loss after Shechita are extensively described by Rosen (2004) and Levinger (1995 and 1961). They state that the heart will continue to beat for a few minutes but after approximately one minute, lack of venous return to the heart leads to reduced cardiac preload and thus diminished cardiac contractility, nevertheless the heart by the above mentioned authors is said to contribute to the bleeding process.

Gregory et al. (2010) described the loss of posture of adult cattle after slaughter without stunning in upright restrained position as follows: When the cattle were released from the head restraint, most stepped backwards, stood for varying lengths of time, swayed or became unsteady and then either fell to one side and slid down the wall or their hind limbs buckled and they fell backwards followed by loss of support from the forelimbs. When down, some animals sat in sternal recumbency, but most fell into lateral recumbency or were leaning laterally. Loss of posture happened average 19.5 seconds post cut (median 11 s, maximum 265 s, Gregory et al., 2010).

Blackmore (1984) reported loss of ability to stand for sheep up to 4 seconds and for calves up to 40 seconds after satisfactory cut, but up to 385 seconds for calves with one carotid occluded. Loss of coordinated attempts to rise took more time (up to 10s for sheep and up to 47s for calves with satisfactory cut, up to 385s for calves with carotid occlusion). It is important to mention though that these times were recorded after severance of exteriorised vessels and not after a full ventral cut. After ventral neck cut one calf was investigated which lost ability to stand after 5 seconds but lost coordinated attempts to rise only 40 seconds post cut (Blackmore, 1984).

In papers originating from Germany during the beginning of the 20th century it is reported that a mature cow lay down in an upright position 20 seconds after ventral neck cut, but after 90 seconds still made righting attempts and looked around. A 3 year old bull lay down 2.5 minutes after a ventral neck cut, still looking around with its head raised and attempted several times to get up thereafter (Hoffmann, 1900).

In 1913 a veterinarian and director of a slaughter house and seven of his colleagues documented some slaughter experiments without stunning photographically and by film. He performed a ventral neck cut in a tied down young cow and a tied down oxen and untied them while they were being cut. The cow made righting attempts directly after the cut, managed to rise 9 seconds post cut, and then walked 5 metres whilst bleeding heavily. Its forelegs straddled 19 seconds after the cut, after 7 more seconds it came down in sternal recumbency having stumbled back another 2 metres, still in sternal recumbency with its head propped up 8 seconds later. The cow fell on its side, and began to convulse after 6 more seconds. Hence convulsion began 40 seconds after the cut. The oxen also rose after the cut. Its head was hyperextended with a gapping bleeding neck wound. The animal then fled 10 metres across the yard, 20 seconds post cut the forelegs began to straddle, and it finally collapsed 7 seconds later. A mature sheep was cut in an upright position, afterwards it jumped forward and walked straight on for 7 metres with its head raised. Thirteen seconds after the cut its forelegs straddled and it collapsed, still holding its head upright for 8 seconds. Six seconds later it pillowed its head on the ground and finally fell on its side after a total period of 31 seconds after the cut (Klein, 1927). These studies were queried by Levinger (1961) because the cut was not a standardized Shechita cut (severance of both carotids was doubted) and in upright animals the blood flow can be inhibited if the wound is not held open.

Levinger (1976 and 1961) describes for recumbent cattle a motor resting phase within the great majority of animals starting directly after the cut and ranging between 8 and 150

seconds (average 35s, n=32). Hoffmann (1900) also noticed a rest phase after ventral neck cut of cattle in lateral recumbency, during which he considered the cattle unconscious (no corneal reflex), because of the sudden blood loss. He stated however that after 20 seconds at the most animals could regain consciousness. They then began to express defence movements, righting attempts and even succeeded to escape when the bonds broke. The view of Hoffmann supports the above mentioned issue of "drifting in and out of consciousness" and the regulatory capacity of the circulatory system after haemorrhagic shock.

Movements during or after the cut may either be conscious reactions or unconscious reflexes. Animals may move as a reaction to pain or unease because of loss of blood pressure and oxygen in the brain. Movements may also be escape reactions. If the spinal cord is touched by the knife, conscious movements of the legs can be a consequence of pain due to scratching on the spinal bone or severing the spinal cord tissue by a metal blade. A period of violent unconscious physical activity can be due to the loss of inhibitory impulses of higher centres with progressing hypoxia as in decerebrated animals. Levinger (1961) reported on the motor reactions in 150 sheep and cattle after Shechita, whereby 6 animals tried to correct the position of their heads. Three of them succeeded to bring it into normal upright position. One sheep with only one carotid cut could regain posture completely and stood upright for some seconds shaking. For the others he could only suggest bad cutting quality but it was not possible to check the cut for all animals.

Brain stem activity expressed by eye reflexes could be evoked for up to 200 seconds in lambs and adult sheep, for up to 330 seconds in calves and for up to 410 seconds post cut in a bull (Blackmore, 1984). In calves after ventral neck cutting or a lateral stab incision (caudal to larynx and dorsal to trachea and oesophagus) a positive corneal reflex was recorded until 90 to 320 seconds (Newhook and Blackmore, 1982a). Levinger (1961) could provoke the corneal reflex in cattle until 20 to 90 seconds after Shechita (average end of positive corneal reflex: 38.8s, n=10). In goats the positive reflex ceased immediately or at the latest 7 seconds post cut (average: 3.4s, n=10). Nine out of 10 sheep lost their positive reflex 10 seconds post cut, the recent 11 seconds post cut (Levinger, 1961). During the Dialrel WP2 spot visits continuous testing of corneal reflex was seldom possible, thus times described are snapshots and do not include any time limits. Corneal reflex could be punctually triggered between 35 and 150 seconds in cattle and between 30 and 120 seconds in sheep or goats respectively. Animals which had to be re-cut seemed to preserve positive corneal reflex for longer time.

Breathing activity like respiratory gasps were reported up to 220 seconds and 420 seconds post cut in sheep and in a bull respectively. While regular breathing was expressed by those calves which did not exhibit the final convulsions for nearly 12 minutes, afterwards the animal were shot by captive bolt to end the experiment (Blackmore, 1984). In another experiment, duration of respiratory gasps in calves was recorded for 190 to 420 seconds following ventral neck cut and lateral stab incision. The authors recorded as well spontaneous vocalisation in one calf out of 8 for more than 3 minutes after slaughter (Newhook and Blackmore, 1982a). However this animal could not have been subjected to a full ventral neck cut, as in this case the trachea would have been severed, making vocalisation impossible. Schulze et al. (1978, original report by Hazem et al., 1977) described regular breathing in a calf between 77 and 185 seconds after Shechita. Another calf, which had to be re-cut after 24 seconds due to decreased blood flow, showed regular breathing between 80 and 148 seconds after the first cut (Hazem et al., 1977). According to Levinger (1961), after the motor rest phase, decelerated and deepened breathing started between 12 and 55 seconds after the Shechita cut and ceased between 55 and 150 seconds post cut (n=10 cattle). The air passed through the cut end of the trachea but breathing was visible by movements of the nostrils.

During Dialrel WP2 spot visits regular but deep breathing could be observed until 255 seconds post cut in single Halal slaughtered cattle. For Halal slaughtered sheep regular

breathing ended between 22 and 90 seconds post cut, whereby it lasted for overall 5 to 50 seconds. In a plant with very skilled staff regular breathing returned for two third of the sheep and stopped on average 35 seconds post cut (n = 90). Gasps occurred after the end of regular breathing but also for those animals, which did not express regular breathing.

Eye reflexes after slaughter without stunning as well as respiratory gasps do not indicate consciousness as they can still be present when there is an isoelectric EEG (Blackmore and Newhook, 1983; Newhook and Blackmore, 1982a). Regular breathing can be present as well in unconscious animals. Nevertheless it may signify that the threshold towards consciousness is not that far away. Regular breathing can also indicate resurgence of consciousness, if bleeding was insufficient.

The time interval between the cut and final full dilatation of pupils as an indicator for brain death was measured between 56 and 114 seconds for sheep and between 200 and 435 seconds for calves, with satisfactory severance of exteriorised vessels. In calves the interval was between 430 and 455 seconds with severed but fully or partly occluded exteriorised vessels. In a calf and a bull the interval was between 140 and 415 seconds after ventral neck cut (Blackmore, 1984). Before the final pupil dilatation, nystagmus of the eyeball could be noticed. For example during a Dialrel spot visit, nystagmus was noticed in cattle 97 seconds after the cut, and in some animals there was turning back of the eyeball. The meaning of both of these signs in relation to consciousness cannot be clearly defined.

Onset of hypoxaemic clonic convulsions was measured subsequent to definite pupil dilatation between 68 and 158 seconds post cut in sheep after severance of exteriorised vessels and at 160 and 440 seconds post cut in a calf and a bull after ventral neck cut (Blackmore, 1984). According to Levinger (1976) convulsions started on average 28 seconds after the Shechita cut (range: 15 to 60 seconds) and lasted between 150 and 240 seconds. Anoxic convulsions, caused by loss of inhibitory influences from higher centres of the brain operating in the caudal reticular formation occur when ischaemia or hypoxaemia are induced resulting in isoelectric EEG or when the brain is disconnected from the body (e.g. following slaughter or decapitation) (Gregory, 1987a). In meat chicken after kosher slaughter Barnett et al. (2007) observed the final convulsions very close to loss ability to retain a standing posture.

In the Dialrel WP2 spot visits onset of clonic convulsions was recorded between 72 and 173 seconds post cut in cattle and between 90 and 120 seconds post cut in sheep and goats respectively. Sometimes no convulsions were noticed but only a stretching or shivering movement of the body. Anecdotal experience of slaughtermen reflects that animals with very pronounced muscles tend to show stronger movements than others.

The major challenge for the evaluation of the post cut period with regard to animal welfare is to define clear clinical indicators for the time point where animals become irreversibly unconscious after slaughter without stunning. Many parameters are used to describe consciousness and unconsciousness (see chapter 2.6 and table 1), but only few can be applied for slaughter without stunning. Time to loss of posture for example will not be of value for animals in recumbent position and animals in close restraint. As for the evaluation of stunning, it will be necessary to describe as well a standard for optimum slaughter without stunning as indicators for missing this standard. Concerning activity of the brain stem like rhythmic breathing or positive reflex responses, scientific evidence gives certain patterns but the temporal sequence cannot be defined clearly for the setting of standards to date. However if rhythmic breathing does not cease at all, this will indicate failure of quick and permanent loss of consciousness after slaughter without stunning. The same will apply for other brain stem reflexes as well.

Clinical indicators for consciousness are coordinated attempts to rise or to regain normal body posture or if the animal's eyes focus on stimuli from the surrounding and follow them (eye

tracking of movements). This is often accompanied by repeated spontaneous closure and opening of the eyelid. Veterinarians of the Dialrel consortium have noticed the following signs during the post cut period:

- attempts to rise or to regain normal body posture,
- attempts to walk,
- delay in falling down,
- reactions to back up cuts or manipulation of the wound edges (e.g. retraction after touching parts of the restraining device),
- reaction to a hand touching the head,
- licking the nose,
- eye tracking of movements often with repeated spontaneous blinking.

Further scientific studies should also include cognitive responses that have been investigated by Limon et al. (2010) as "response to threat stimulus". This was done by rushing the hand towards the eyes and observing if the animal reacted by closing its eyes and also by moving the head backwards or " responses to different odours or flavours" when introducing a stick in front of the nostril or in the mouth, which was positively answered when the animal's nostrils flared or there was tongue movement.

In conclusion, after the ventral neck cut has been performed on the animal, clinical signs may include reactions to previous manipulations, signs of fading consciousness and weakness or indicators for resurgence of consciousness. Thus it is difficult to define the exact moment of change between the conscious and the unconscious state. Clear signs of consciousness are "attempts to rise or to regain normal body posture", "coordinated reactions to manipulation of the wound edges" or "the animals' eyes focussing on stimuli from the surrounding and following them, which is often accompanied by repeated spontaneous blinking". If these signs are expressed or if rhythmic breathing does not cease at all, this will indicate failure of quick and permanent loss of consciousness after slaughter without stunning.

4.2 Stunning prior to neck cutting

Effective stunning will remove the risk that the animal will experience pain and distress during slaughter and subsequent bleeding. Additionally in a stunned animal the cut will be easier to perform. This helps to sever the blood vessels more accurately and achieve a rapid bleed-out especially in large livestock (Gregory, 1998c).

Efficient stunning methods disrupt the neurons and neurotransmitter regulatory mechanisms in the brain. Since the intention of humane slaughter regulations is to avoid or minimise anxiety, pain, distress or suffering at slaughter, stunning methods should ideally induce immediate and unequivocal loss of consciousness and sensibility. When loss of consciousness is not immediate, the induction of unconsciousness should be non-aversive. The potential duration of unconsciousness induced by a stunning method should be appreciably longer than the sum of the time interval between stunning and sticking plus the time it takes for blood loss to cause death. Sticking should therefore be performed quickly after the stun and, in this process, the major blood vessels supplying oxygenated blood to the brain must be severed to ensure rapid onset of death (EFSA, 2004, page 26 ff.).

So called "reversible stunning methods" allow the animals to regain consciousness, if bleeding was not performed. For these methods it is vital that sticking is performed immediately and effectively to achieve rapid and sufficient blood loss to prevent resumption of consciousness. It is also an important requirement that animals showing signs of return of consciousness must be re-stunned immediately using an appropriate back-up method. With so called "irreversible stunning methods" the majority of the animals concerned would not regain consciousness even if sticking was not performed. Nevertheless death will often be

achieved by blood loss following slaughter and not by the stunning method itself, because blood loss from sticking has a more immediate effect on the brain. In the following sub-sections the focus is on stunning methods which are applied during religious slaughter.

Table 6: Stunning methods used in the context of religious slaughter

Species Stunning method	**Cattle**	**Sheep and goats**	**Poultry** (chicken and turkey)
Electrical head only stunning	**X**	**X**	**X**[1]
Penetrating captive bolt stunning	**X**	**X**	**X**[2]
Non-penetrative captive bolt stunning	**X**	**X**[2]	**X**
Gas stunning			**X**

[1] Current also applied through the whole body but without induction of ventricular fibrillation

[2] Not in general use

4.2.1 Electrical stunning

Electrical stunning causes unconsciousness and insensibility by producing a depolarisation shift in nerve cells followed by hyperpolarisation of action potentials which leads to epileptiform discharges (Gregory, 1987b). If an electric current is applied to the head, and sufficient current flows through the brain, unconsciousness occurs in a similar manner to that produced in grand mal epileptic seizure. There is disordered metabolism and electrical activity, which cannot support conscious activity (Gregory, 1998a). Grand mal epilepsy is a pathological extreme of neuronal synchrony and is considered to be incompatible with normal neuronal function and, hence, persistence of consciousness (Cook et al., 1995; Cook et al., 1992; Hoenderken, 1978).

The electrical current flow through the head has been considered painful (Rosen, 2004), though Levinger (1976) admits that in most cases where electric shock therapy is used in humans the patient loses consciousness before he feels pain. It is true that a poor initial contact, a slow rise in current levels or insufficient peak current levels, may not stun the animal immediately and it could be experienced as a painful electric shock. However it is known, that when electrodes are properly placed with the necessary minimum current that within far less than one second synchronization of electric potentials in the brain is achieved. This disrupts all coherent processing of information by the brain before electric shocks can be sensed as painful. After the current has been maintained for a given time the nerve cells are unable to react to further stimulation in a way that can be associated with consciousness (Gregory, 1987b; Hoenderken, 1978; Warrington, 1974).

The effects of the current flow at the neuro-chemical cellular level are now well understood. Neurotransmitters enable the communication between neurons in the brain. Excitatory (Glutamate/Aspartate) and inhibitory (GABA) neurotransmitters interact to form a physiological equilibrium. After the electrical current flows through the brain there is a dramatic rise in the extracellular concentrations of Glutamate and Aspartate. The cell structures are in a state of heightened excitation and uncoordinated activity. The interruption in the processing of signals leads to an immediate loss of consciousness (within 200 ms (Cook et al., 1995)). The slower release of GABA during the course of the epileptiform fit brings the fit to an end. Because elevated concentrations of GABA can also be due to stress and elevated GABA levels can inhibit the effects of Glutamate/Aspartate, stress can negatively influence the ability to produce epilepsy. This illustrates the importance of careful handling of animals prior to stunning. The elevation of the extracellular concentration of GABA lasts significantly longer than that of Glutamate/Aspartate and contributes to a long lasting analgesia after the electrical stun (5-15 min). The role of the neurotransmitters in electrical stunning was clarified in earlier experiments with pharmacological agonists and antagonists. These findings

were recently confirmed through real time studies using microdialysis probes. It was concluded that electrical stunning is a humane method of rendering an animal instantaneously unconscious and with timely and effective bleeding unconsciousness and insensibility will last until death supervenes by bleeding (Pleiter, 2005; EFSA, 2004; Cook and Devine, 2003).

Two kinds of electrical methods are used at present (EFSA, 2004, page 34):
- Electrical head only stunning: involves transcranial application of an electric current in red meat species and poultry, in the latter however the current can also be applied through the whole body (waterbath).
- Electrical head to body stunning: usually involves head-to-body application of an electrical current in red meat species and poultry.

Depending on electrical frequency that is used, the latter method can produce ventricular fibrillation in the heart and thus lead to a prompt and sustained decrease in blood pressure and avoid resumption of consciousness if bleeding is too late or badly performed (Gregory and Wotton, 1984b). In the context of religious slaughter, head electrical stunning methods would be used, where loss of consciousness is reversible, but there will be a risk of resumption of consciousness during bleeding if bleeding efficiency is poor.

Ventricular fibrillation will depend on: 1. the pathway the current takes through the body; 2. the region of the heart that receives the current; 3. the phase of the heartbeat cycle which coincidences with the start of the current; 4. the duration of current flow; 5. the frequency and waveform of the electrical current (high current frequencies are less likely to induce a ventricular fibrillation (Gregory et al., 1991)); 6. the species, as the heart in species with high intrinsic heart rates is less readily fibrillated with electrical currents (Gregory, 1998a). Thus by influencing the current parameters and pathway, ventricular fibrillation can be avoided. For secular slaughter, ventricular fibrillation is used to achieve a more secure stunning effect while overall bleed-out will not be influenced (Raj and Johnson, 1997; Gregory and Wilkins, 1989a). Another advantage of induction of ventricular fibrillation or cardiac arrest will be that the expression of bruises inflicted at stunning or slaughter will be reduced (Gregory et al., 1988b; Gregory and Wilkins, 1984), though the efficiency of bleeding is still the overriding measure to prevent haemorrhagic conditions in carcasses (Gregory and Wilkins, 1989b). As ventricular fibrillation or cardiac arrest present a risk of being painful to the animals, it is essential to stun the animal before or at the same time as inducing cardiac arrest (Gregory, 1998a).

When an animal is electrically stunned by head-only stunning there is a prompt fall in heart rate whilst the current is flowing, but when the stunning current is switched off heart rate rapidly rises to above normal rates (Gregory, 1998a).

During the current flow through the brain, the body of the animal becomes rigid, because brain stimulation and electrical impulses passing down the spinal cord cause tonic muscle contraction. The hind legs are flexed and if its weight is not mechanically supported, the animal would fall to the ground. When the current flow stops, the generalized tonic contraction usually continues for a short period (tonic phase, e.g. 10 seconds) and then convulsions (clonic phase) set in. These convulsions are driven by dysfunction of certain brain structures, e.g. reticular formation (Gregory, 1998a). Tonic muscle contraction during current flow as well as tonic and clonic activity after current flow are important clinical signs which can be used to diagnose that stunning has been performed correctly (Wenzlawowicz, 2006; EFSA, 2004, page 36 ff.). A useful behavioural sign is to watch for return of rhythmic breathing, as these coincide with the end of epileptiform activity in the brain and often as well with the end of carcass kicking. The return of rhythmic breathing indicates that hypersynchrony of brain neurons has ended and some of the normal function has been restarted. Other functions will probably follow, and resumption of consciousness is

impending. If there is no or insufficient bleeding after electrical stunning, without induction of ventricular fibrillation, animals will start rhythmic breathing and will soon regain consciousness (Gregory, 1998a).

Following (EFSA, 2004, page 41) the signs of a successful electrical stun are:
- Immediate collapse of free-standing animals (not be applicable to poultry restrained in a cone or shackle or animals held in a restraining conveyor);
- Immediate onset of tonic seizure (tetanus) lasting several seconds, followed by clonic seizure (kicking or uncoordinated paddling leg movements), applies to all red meat species and to short duration water bath electrical stunning of poultry. Head-only electrical stunning of poultry leads to clonic-tonic convulsions (a reverse of sequence seen in red meat species);
- Apnoea (absence of breathing) lasting throughout tonic-clonic periods;
- Upward rotation of eyes (except for poultry).

Indicators of failed stunning are escape behaviour often with vocalising, absence of the typical tonic or clonic muscle activity, resumption of rhythmic breathing, vocalisation during and after the current application or righting attempts after current application. If the eyeball is able to focus and follow stimuli from the surrounding, the animal is conscious (EFSA, 2004; Aichinger, 2003).

For effective electrical stunning the necessary technical requirements have to be fulfilled under practical conditions. This includes the issues of performance (e.g. sufficient restraint, application of the electrodes with correct pressure, within the required time, at the correct position to span the brain). Correct and timely sticking and in case measures to facilitate electrical contact are necessary (e.g. moistening the skin). Equipment must be fully functional, this includes well maintained suitable electrodes that provide adequate opening. The equipment must be clean and not corroded to achieve good electrical contact, an undamaged transformer and well isolated cables delivering a current of sufficient amperage and the right waveform are essential. The settings of the stunning apparatus must be monitored and corrective measures must be taken if necessary (Wenzlawowicz, 2006; EFSA, 2004, page 51). Conditions for electrical stunning in each species are described by EFSA (2004).

In cattle and calves the major challenges with head-only electrical stunning are the short duration of the epileptiform insult and the occurrence of strong clonic convulsions. Various studies have shown that the duration of unconsciousness, measured from the resumption of normal breathing, was between 20 and 90 seconds. Effective bleeding must be achieved within this period to avoid resumption of consciousness. As pre-thoracic sticking induces a dramatic blood pressure loss within 8 seconds and evoked responses were not present after 5 seconds in calves (Anil et al., 1995a), simple calculation of 20 minus 8 seconds suggests that pre-thoracic sticking should be carried out within 12 second after the stun. Thus rapid pre-thoracic sticking resolves the problem of short duration of unconsciousness after electrical head only stunning. In Australia and New Zealand pre-thoracic sticking immediately after the Halal cut is routinely practised to avoid problems of regaining consciousness but also carcass quality problems, which could arise if bleeding is impaired (Pleiter, 2005).

Scientific evidence resulting from analysis of EEG and neurotransmitters indicates that correct head only electrical stunning followed by neck cutting within 10 seconds is an effective method. Both, stunning and neck cutting additively increase the respective neurotransmitters in the brain, which implies that electrical stunning accelerates brain failure after sticking due to its exhaustive effect on brain metabolism (Cook and Devine, 2003; Cook et al., 1996; Cook et al., 1995; Bager et al., 1992; Devine et al., 1987; Devine et al., 1986b). Investigations of the first two authors within Dialrel WP2 involving head only stunning of 80

adult cattle in a turning pen, revealed that effective stunning and permanent insensibility can be achieved with precise electrode placement plus a skilled cut performed within 5 to 8 seconds after the end of a 4 seconds current flow. During the cut the animals were still well restrained in the rotary pen. Thus the second problem mentioned above – the clonic convulsions – can be managed by means of restraint and immediate cutting, or by electro-immobilisation with low voltage spinal discharge (Wotton et al., 2000; Devine et al., 1987; Devine et al., 1986b). However this may mask potential signs of returning consciousness.

The recommended minimum amperage is 1.5 amperes for adult cattle and 1.3 amperes for calves up to 6 month of age. In practice depending on the construction and placement of electrodes often 2 to 3 ampere are applied in cattle. Voltages used are 350 to 400 Volts. Electrode position for handheld tongs is preferably temporal between the eye and the ear. With automatic current application the current flows through the brain between neck electrodes and a nose plate. Current should be applied for at least 4 seconds to the head (EFSA, 2004, page 70). If ventricular fibrillation is to be induced at least 1.5 amperes are recommended for cattle and about 1.0 ampere for calves, applied for minimum 5 seconds, but in practice again often higher currents and longer application times are used (EFSA, 2004, page 70).

Under routine conditions effectiveness of electrical stunning however may be low, due to technical shortcomings. Aichinger (2003) reported that 10 percent of 619 cattle stunned by a Jarvis beef stunning device with automatic application of a 4 second head current and subsequent induction of ventricular fibrillation were re-stunned by captive bolt. Although the staff tended to re-stun cattle which could be considered unconscious the percentage of cattle showing focused eyeball movements and regular breathing was due to very late sticking after shackling. In another investigation of head only electrical stunning 9 of 23 cattle were considered insufficiently stunned by showing eye tracking of stimuli and coordinated leg movements. In this case the cause was presumably a low voltage of only 250 Volts and bad sticking quality was presumably the reason (Stueber, 2000).

In sheep and goats the main principles apply as for cattle, as described by EFSA (2004, page 77 ff.) and Blackmore and Delany (1988). The tongs should be positioned between the eyes and the base of the ears on both sides of the head preferably on local wet skin. Wool, dry skin and placement of the tongs in a caudal position behind the ears, lowers stunning effectiveness (Velarde et al., 2000). Pointed electrodes (electrodes with pins) give good grip and electrical contact, because they penetrate the wool. Electrodes with serrated edges may work in shorn sheep and if the area of application is moistened. With small areas of contact between the sheep's head and the electrodes, wool-burning and marked carbonising of the electrodes can occur. This, in turn, leads to a poor electrical contact due to an increased electrical resistance in the pathway and special care is necessary to keep the electrodes clean.

Effective head-only stunning in sheep should be induced using minimum currents of 1.0 Ampere. A minimum of 250 Volts should be used to deliver the current. Duration of current flow should be a minimum of 2 seconds. The maximum stun-to-stick interval is 8 seconds (EFSA, 2004, page 78). Following anecdotal reports for mature sheep even higher intensities of current about 1.3 to 1.5 Amperes may be necessary to achieve sufficient stunning effectiveness. Currents used in sheep in practice often have a higher frequency than 50 Hertz, e.g. 100 or 400 Hertz and also current patterns are used where the frequency decreases during current application from 500 Hertz to 100 Hertz.

In order to check for clinical signs of correct stunning and recovery in sheep, the safest indicators are the typical pattern of seizures and return of normal rhythmic breathing. Resumption of rhythmic breathing can occur during the second clonic phase, as in lambs the

seizure activity after high voltage head-only stunning includes a tonic and two clonic phases (Velarde et al., 2002).

Anecdotal experience of the Dialrel WP2 members revealed that in examples of bad routine practise, between 4 and 20 percent of sheep may be ineffectively stunned because of wrong positioning of the electrodes. This is often due to inadequate restraint in relation to slaughter speed, too short application time of current and late sticking.

In poultry two stunning methods are used in the context of reversible electrical stunning: head-only electrical stunning, where the current is applied only to the head via a pair of electrodes, and electrical water bath stunning where high frequency currents are used that do not induce cardiac arrest. For both methods the depth and duration of unconsciousness depends upon the amount and frequency of currents applied (EFSA, 2004, page 116 ff.).

The frequencies used in modern electrical stunning systems of poultry ranges from 50 to 1500 Hertz. Waveforms of currents are pulsed direct currents (DC) and sine wave alternating currents (AC). As a consequence of the variations in waveform and frequency, measurement of current amperage has become sophisticated. Depth and duration of unconsciousness induced with all the waveform frequency combinations used in practice, have to be determined based on sound scientific studies with special regard to the variable conditions in practice (EFSA, 2004, page 120; Wenzlawowicz and Holleben, 2001; Gregory and Wotton, 1986).

High frequency electrical stunning is important in poultry stunning, because it leads to a more even and less pronounced contraction in the muscles and thus helps to prevent blood blemished meat. Other possible carcass downgrading like broken bones and red wing tips are also reduced. The disadvantage of high frequency stunning is, that a shorter lasting stunning effect is produced, especially in the quickest recovering birds (Mouchoniere et al., 1999; Wilkins et al., 1998; Hillebrand et al., 1996) and extra care is needed in checking that birds remain insensible throughout the bleeding period (Gregory, 2007).

Poultry, unlike red meat species, do not show grand mal epilepsy in the brain following electrical stunning. Nevertheless scientific literature suggests that the electrical stunning-induced release of monoamines and inhibitory amino acid neurotransmitters in the chicken brain may play a prominent role in the induction and maintenance of unconsciousness following electrical stunning (Raj, 2003). These mechanisms would also appear to be relevant to the manifestation of a profoundly suppressed EEG and abolition of SEPs following electrical stunning, which are suggested to be meaningful indicators of an effective electrical stun in chickens. Research to date indicates that electrical stunning indicative of unconsciousness in chickens should lead to a period of spike-and-wave epileptiform activity. This is followed by a period (of at least 30 seconds) of profoundly suppressed or quiescent EEG immediately thereafter, indicative of spreading depression or neuronal fatigue in the brain (Schütt-Abraham et al., 1983a).

Return of eye reflexes and normal breathing are useful indicators of the early return of brain function after electrical stunning of poultry. Whereas a reaction to comb pinching or return of neck tension are not considered useful, as their return appears to be very late relative to other functions. During bleeding vocalisation and wing flapping must be absent as well as head raising, spontaneous blinking and directed eyeball movements (focused on stimuli from the environment) (Wenzlawowicz and Holleben, 2001; Schütt-Abraham, 1999).

Head only electrical stunning is used commonly to stun poultry on the farm and as a back-up method. For head-only stunning the birds are restrained by hand, in a cone or shackles, between the legs or in a crush and the current is delivered by a pair of tongs or fixed

electrodes, into which the head of the chicken is manually introduced (Wenzlawowicz et al., 2006).

Head-only electrical stunning induces flexion of legs followed by leg extension and wing flapping from the moment of current flows across the head. These are followed by tonic seizures as indicated by stiffening and arching of the neck, rigid extension of the legs, wings folded tightly around the breast and muscle tremor. During tonic seizure, eyes will be wide open (no blinking when touched) and rhythmic breathing will be absent. Return of eye reflexes and normal breathing precedes a return of consciousness (EFSA, 2004, page 120). One problem following head only stunning is the presence of severe wing flapping. This can impede prompt neck cutting. It can be addressed by either by prolonging the current flow through the brain or applying a so-called high frequency relaxation current through the spinal cord (Raj and Tserveni-Gousi, 2000; Hillebrand et al., 1996).

A minimum current of 240 milliamperes for chickens and 400 milliamperes for turkeys should be applied to the head for at least 7 seconds, when using a constant voltage stunner (110 V) supplied with 50 Hertz alternating currents. Neck cutting must be performed within 15 seconds from the end of the stunning current (Gregory and Wotton, 1991; Gregory and Wotton, 1990a). With constant current stunners and low impedance electrodes, minimum currents increase with increasing frequency from 100 milliampere for 50 Hertz and 150 to 200 milliampere for 400 and 1500 Hertz sinusoidal alternating currents respectively, to be applied for 4 seconds with neck cutting also within 15 seconds (Raj and O' Callaghan, 2004).

Waterbath electrical stunning is the most common electrical stunning method used for poultry, and requires upside down restraint in shackles. In a waterbath the current flows from the bath through the birds to the earthed shackle line. Although the current passes through the whole body, waterbath stunning can be performed as a reversible stunning method, if the combination of current parameters (e.g. amperage and frequency) does not induce ventricular fibrillation. Ventricular fibrillation and cardiac arrest will be markedly reduced if current frequencies increase above 300 Hertz (Gregory et al., 1991).

In multiple bird waterbath stunning systems, the current flows through all birds at the same time, and the animals' electrical impedances form a parallel circuit. In this arrangement it is possible that some birds receive more current than others, because the electrical impedance varies between birds (Raj and Tserveni-Gousi, 2000). This problem will be less important, if good and uniform electrical contact can be provided between the shackles and the birds legs, e.g. by moistening the shackle-leg contact point, and if the birds are uniformly immersed.

For waterbath stunning it is very important to follow the technical requirements to achieve effective stunning without unnecessary pain and suffering. The negative impacts of shackling can be minimized as far as possible by limiting shackling time, using the appropriate size of shackle, prevention of wing flapping (e.g. by using a breast comforter belt and using blue light). There must be secure and uninterrupted contact between the shackle and the earth (rubbing) bar. The height of the water bath must be adjusted according to the size of poultry. The electrodes in water bath stunners must extend to the full length of the water bath. There must be provisions such as electrically isolated entry ramps at the entrance to the water bath to prevent pre-stun electric shocks. For the same reasons the water must not overflow at the entrance of the bath. Birds' heads must be completely immersed in the water bath, preferably up to the base of their wings. Electrical devices must display visibly the total voltage and current delivered to the water bath and these should be appropriate to the waveform of the current used (EFSA, 2004, page 133 ff.; Schütt-Abraham, 1999).

Voltage must be sufficient to ensure that every bird in the bath receives the recommended minimum current. Based on the existing scientific knowledge, it can be suggested that the minimum current necessary to stun chickens would be 100, 150 and 200 milliamperes per bird

in a water bath supplied with up to 200, above 200 and up to 400, and above 400 and up to 1500 Hertz sinusoidal alternating current, respectively. For turkeys the minimum currents for the same frequency ranges were given as 250, 400 and 400 milliamperes. When currents of lower than this are applied, the depth and duration of unconsciousness induced by the stun may not be adequate to prevent resumption of consciousness before neck cutting or during bleeding (EFSA, 2004, page 134). For turkeys of 6 kilograms liveweight only, Mouchoniere et al. (2000) achieved acceptable stunning quality with a 150 milliamperes 300 Hertz sinusoidal alternating electrical current applied for 4 seconds.

Where high frequency stunning is performed in a waterbath, neck cutting must be performed within 20 seconds from the end of stunning and both the carotid arteries in the neck must be cut (EFSA, 2004). Practical experience shows however that effective stunning in many plants can only be achieved if sticking is performed within 5 to 10 seconds. This may be due to the fact that severing both carotid arteries is not given enough importance and in practice only one external jugular vein or the vertebral arteries are severed at the back of the neck.

To summarize waterbath stunning, if properly performed, can be an efficient method of stunning. However the disadvantage of shackling persists and there are several welfare risks like pre-stun electrical shocks and insufficient stunning and bleeding. In practice bad stunning effectiveness is often overlooked, because currents may also cause immobility only without achieving unconsciousness and the used current parameters are not known or not properly displayed. Sometimes current parameters have been changed because of experience with carcass quality defects, whereas it would have been more effective to improve the quality of neck cutting and decrease the time interval thitherto. According to the experience of the Dialrel WP2 members, stunning failures in poultry slaughter plants using electrical waterbath stunning in the worst cases involved 10 to 15 percent of the birds and was due to insufficient current or late sticking.

Negative impacts of electrical stunning on carcass and meat quality can occur with high voltages, long current flow durations, repeated application of current, wrong electrode placement, bad electrical contact, in animals with a predisposition to haemorrhage formation (e.g. raised capillary fragility in young lambs or broilers) and engorgement of the capillary bed at the time of slaughter. They can however be minimized by managing the above named impacts, good handling, use of constant current stunners, application of high frequency currents, producing ventricular fibrillation at stunning and most importantly by prompt and effective sticking (Gregory, 2007).

To conclude electrical stunning is a humane method of rendering an animal instantaneously unconscious and with timely and effective bleeding unconsciousness and insensibility will last until death supervenes by bleeding. Nevertheless, it must be guaranteed that the necessary technical requirements are fulfilled under routine conditions and welfare can be poor in case of noncompliance.

4.2.2 Mechanical stunning - penetrating captive bolt stunning

Among mechanical stunning methods the penetrating captive bolt method, has to be distinguished from the non-penetrating method (see chapter 4.2.3). The non-penetrating method is sometimes called "concussive stunning", though concussion is the underlying principle for both methods. During penetrating captive bolt stunning there is structural damage to the brain in addition to the concussive impact on the skull.

Both types of gun are normally fired on the forehead (usually frontal bone) of an animal, but other sites may be selected when there are horns or thick ridges on the skulls. Captive bolts must always be fired perpendicular (at right angle) to the skull bone surface (at the chosen site); otherwise bolts may skid and fail to fully impact the skull.

With <u>penetrating captive bolt</u> guns, a steel bolt is powered by cartridges or compressed air, and for poultry the bolt may be spring driven. The bolt is not pointed, but the tip is sharpened in a concave manner and has a sharp rim without nicks. To achieve good stunning the captive bolt device must be correctly placed and a bolt of adequate length and diameter must be sufficiently accelerated. Consequently there will be transfer of energy to the animals head, causing concussion but also structural damage as the bolt travels through the brain. Immediate insensibility and unconsciousness is caused by rapid propagation of shockwaves of kinetic energy through the brain and abrupt acceleration and deceleration of the relatively soft brain within the bony skull (shear and contre-coup effects). This impact can be short lasting. For different species and sizes of animals, different guns are used, these have differing mass, length and diameter of bolts. Specific cartridge strengths or air operating pressure are also used for different animal types and there are specific shooting positions on the animals head for different species. If the bolt is too thin or it is fired through a trephined skull there will not be enough energy transfer to the head to induce effective stunning (Karger, 2009; EFSA, 2004, page 45 ff.; Raj and O'Callaghan, 2001; Daly and Whittington, 1989).

The animal's head must be suitably presented to the operator to facilitate accurate shooting. The animal should be rendered unconscious using a single shot and effective bleeding is required and needs to be performed immediately after stunning to ensure rapid brain death following exsanguination (EFSA, 2004, page 48).

Effective stunning can be monitored from immediate collapse and prompt, persistent absence of rhythmic breathing. The muscles in the back and legs go into spasm, forelegs and hindlegs are flexed, the forelegs straightening after a few seconds. Signs that indicate a shallow depth or concussion include flaccid muscles immediately after stunning, return of rhythmic breathing and rotated eyeballs. Return of rhythmic breathing happens if stunning is insufficient or bleeding is too late (Gregory, 1998c).

Heart activity after captive bolt shooting in cattle can continue for about 4 minutes in animals that are bled immediately following stunning, but it continued for 10 minutes in animals that were not bled (Vimini et al., 1983). Schulze et al. (1978) also found persisting activity of the heart and described an increase of heart rate up to 300 beats per minute after stunning in sheep and calves. According to Kaegi (1988) heart rate and blood pressure increased after captive bolt stunning of cows due to activation of the respective brain centres.

Captive bolt guns can be either trigger or contact fired. With contact fired guns, there is no possibility to correct the position of the gun once it touches the head of the animal. As a consequence there are more failed shots with this type of gun in some plants depending on the skill of the staff. Bad maintenance of guns is often the reason for stunning failures. Guns have to be cleaned and maintained regularly, otherwise the velocity of the bolt may be impaired. Worn rubber rings or springs used to retract the bolt from the skull have to be replaced at once, failure to do so can result in damage to the bolt and gun (e.g. prevent the pistol getting stuck in the skull and becoming damaged). If the tip of the bolt is protruding from the muzzle of the gun, this indicates bad maintenance, this results in enlargement of the gas expansion chamber within the gun and leads to underpowered shots. Deformed bolts will not achieve the necessary speed. Too short or narrow bolts can lead to decreased stunning effectiveness, through decreased kinetic energy transfer (Gregory, 2007; EFSA, 2004, page 45 ff.; Holleben et al., 2002).

Effective captive bolt stunning is associated with immediate absence of evoked cortical responses in the brain (Daly and Whittington, 1989; Daly et al., 1987). Absence of primary cortical evoked responses indicates failure in neurotransmission at a level that occurs before conscious perception of a stimulus. Conceptually this is a useful indicator of a deep stun, as it indicates deafferentation before signals can reach the association cortex, where signals

associated with consciousness are integrated. Unlike evoked responses the spontaneous EEG is not as reliable as an indicator of brain disturbance following captive bolt stunning, and evoked responses are preferred (Gregory, 2007; Daly et al., 1988; Daly, 1987). Schulze et al. (1978) used EEG nevertheless and they described after captive bolt stunning severe changes in the EEG. Whereas after Shechita the EEG remained unchanged for several seconds (see chapter 4.1.2, table 4, 5). But as the isoelectric EEG after captive bolt stunning appeared later than after Shechita they doubted the effectiveness of captive bolt stunning. This interpretation was immediately criticized by Kotter et al. (1979) and would not withstand scrutiny today, because unconsciousness is achieved before the EEG becomes isoelectric. Concerning the comparative study of Schulze et al. (1978) it is worth noting that at that time captive bolt devices have not been sufficiently standardized, which was mentioned by the authors in an answer (Schultze-Petzold and Schulze, 1979). The stunning effectiveness reached by Schulze et al. (1978) at that time obviously did not reach today's standard as breathing was recorded for 3 of the 5 calves, stunned by captive bolt (Hazem et al., 1977).

In cattle, to ensure efficient stunning, the captive bolt must be fired at the crossover point of imaginary lines drawn between the base of the horns and the contralateral eyes and certainly no further away than 2 centimetres radius from this point (EFSA, 2004, page 59; Finnie, 1993; Ilgert, 1985; Lambooij et al., 1983; Lambooij, 1981a; Lambooij, 1981b). Kaegi (1988) gives the outer corner of the eye as reference point to the base of the horns, thus slightly moving the aim upwards. Shooting accuracy becomes more critical using low powered devices (Gregory, 2007). Deviations from the recommended shooting position and from the perpendicular shooting direction increases intensity of muscle spasms after shooting and this may impede further processing including hoisting and sticking (Marzin et al., 2008; Kaegi, 1988; Ilgert, 1985).

As reported by anecdotal experience of the first two authors, return of rhythmic breathing in cattle is a particular concern in very large bulls or cows (> 600 kg). It could often be reduced by prompt and effective sticking, shooting slightly higher than the recommended position or using newer gun models with high bolt velocities or extra long bolts (14 cm). Nevertheless heavy and long bolts have to be equally accelerated and targeting is more difficult with heavy devices. Pneumatically operated captive bolt stunners are also quite heavy and they have to be held by two hands and suspended above the animals head. Close restraint of the animal's head is generally necessary for exact aiming with these stunners, because otherwise the operator cannot follow the animal's head movements and revise the aim.

Bolt velocities have to be above 55 m/s for steers heifers and cows and 70 m/s for young bulls that are usually more difficult to stun. The transfer of energy to the head and the depth of the stun are improved when bolt diameter is 16 mm or more (Gregory, 2007).

Grandin (2003) reported return to sensibility problems in 4 out of 21 commercial plants using both air powered and cartridge fired captive bolt guns. This involved 0.16% of the steers and heifers and 1.2% of the bulls and cows. The problems were due to damp cartridges, poor maintenance of guns, wrong shooting position and cattle with very thick and heavy skulls. In a French plant 7 percent of 500 cattle were reshot because they did not collapse, mostly because of wrong bolt position (Marzin et al., 2008). The authors also noticed righting movements in nearly a quarter of the cattle, however it is not clear whether these can be considered conscious activity, because breathing was not assessed (Marzin et al., 2008). The prevalence of a shallow depth of concussion determined in 1600 cattle from physical collapse, presence or absence of corneal reflex, normal rhythmic breathing, eye ball rotation, and whether the animal was re-shot, was 8 percent for all cattle and 15 percent for young bulls. These figures were recorded from a plant with highly trained sensible staff, who the authors noted probably re-shot animals more than was needed. Arousal of cattle before stunning and soft-sounding shots were associated with less efficient stunning (Gregory et al., 2007). In a

large German slaughter plant 6 percent of 1130 heavy cattle of flecked breed had to be re-stunned (Endres, 2005). According to the experience of Dialrel WP2 partners, insufficient stunning can be as high as 5 or even 15 percent, due to inadequate equipment and improper operator performance (e.g. positioning of the stunner and late sticking especially in heavy cattle). Changes in equipment and employee training contribute enormously to good efficiency in captive bolt stunning of cattle (Gallo et al., 2003).

In summary, captive bolt stunning, when fired with appropriate devices using correct cartridges or air pressure and applied accurately, induces reliably effective stunning in all adult cattle and calves. It is nevertheless very important with this method to reliably re-stun animals when poor stunning is suspected. Bleeding within 60 seconds can pre-empt suffering in cases of slight deviation of optimum performance.

In sheep and goats generally the same principles apply as for cattle. Ideal shooting position for polled sheep is the highest point of the head in the mid-line, pointing straight down to the throat. The ideal shooting position for horned sheep and for all goats is the position just behind the middle of the ridge that runs between the horns. Then the captive bolt should be aimed towards the mouth (HSA, 2006; EFSA, 2004, page 74).

Changing the shooting position from the frontal position to the poll may alter the mechanics of the impact such that the diffuse damage to the brain is reduced, possibly owing to reduced acceleration of the head and can be associated with rapid recovery of brain function in sheep (average: 50 s, earliest: 33 s after the shot). Therefore shooting in the poll position should only be used when it is essential (i.e. in horned animals) and then always followed promptly by sticking within 16 seconds (Daly and Whittington, 1986). Both common carotid arteries should be severed to keep the time to loss of brain responsiveness as short as possible (Gregory and Wotton, 1984a).

Based on practical experience, behaviour post-stunning is very similar to that seen in cattle. There is prompt and persistent apnoea and immediate onset of tonic seizure. The position of the eyeball is fixed, i.e. facing straight ahead (EFSA, 2004, page 75). During bleeding strong clonic convulsions can occur.

Failed stunning can occur due to wrong positioning of the stunner. However when performed correctly, penetrative captive-bolt stunning is an effective method of stunning sheep and goats, and loss of consciousness is immediate.

In poultry mechanical devices which are penetrating as well as non-penetrating have been developed specifically to kill, rather than stun birds (Hewitt, 2000). Because the skull bones are not ossified in poultry, both penetrating and non-penetrative devices induce severe structural damage to the brain and immediate death, provided the bolt parameters are adequate, rather than concussion of the brain (EFSA, 2004, page 151).

Birds are restrained in cones, shackles, crushes or by hand and captive bolts must be fired perpendicular (at right angles) to the frontal bone (Raj and O'Callaghan, 2001). Bolt diameters between 5 and 6 millimetres and a length between 10 and 25 millimetres are reported to be effective in chicken. Visual evoked potentials were immediately lost, but there can be severe wing flapping (Raj and Tserveni-Gousi, 2000; Hillebrand et al., 1996).

To conclude, penetrating captive bolt stunning is a humane method of rendering an animal instantaneously unconscious, provided that the stunning apparatus is well maintained, placed correctly and the correct power of cartridges or air pressure is applied. Effective bleeding must follow the stun. Because deviations from the optimum target area and angle are always possible in routine slaughter, it is nevertheless important with this method to reliably re-stun animals in case they are insufficiently stunned.

4.2.3   Mechanical stunning – non-penetrative captive bolt stunning (concussive stunning)

Depending on the amount of brain damage induced, non-penetrative captive bolt stunning can cause either permanent or temporary unconsciousness. In a study on lambs and calves, the majority showed signs of recovery (Blackmore and Delany, 1988, page 57). These signs and the development of righting reflexes did not usually occur in less than 2 minutes (Blackmore, 1979).

To ensure effective stunning in adult cattle, the non-penetrative captive bolt must be placed 2 centimetres above the cross-over point of imaginary lines drawn between the base of the horns and the contralateral eyes. This must be achieved very precisely using proper body and head restraint, because only slight variation in the ideal shooting position and angle decreases stunning efficiency (HSA, 2006; Grandin, 2003; Hoffmann, 2003). Endres (2005) suggests that massive hair on the forehead or moulding of foreheads hinders good contact of the concussive head to the bone and thus lead to decreased energy transfer.

Studies on effectiveness of the method reveal different results. Finnie (1995) after studies on 12 adult cattle found that frontal non-penetrative captive-bolt-stunning resulted in immediate loss of consciousness in all animals, as indicated by immediate collapse and absence or rhythmic breathing. Whereas Lambooij et al. (1981) using electroencephalographic methods could only produce immediate unconsciousness in 15 out of 19 veal calves of 200 kilograms live weight. Blackmore (1979) found that 80 percent of 90 calves between one and 2 weeks of age were effectively stunned as determined by behavioural observations.

Gibson et al. (2009d) tested the electroencephalographic (EEG) and cardiovascular responses of halothane-anaesthetised calves (109 to 144 kg live weight) to non-penetrative captive-bolt stunning and showed that non-penetrative captive-bolt stunning virtually instantaneously altered cerebrocortical activity. Immediately after stunning, respiration ceased in all calves. Some animals exhibited slow uncoordinated limb movements during the first 5 seconds. The frontal bone of all calves had a 30-millimeters diameter circular depressed fracture at the site of impact of the bolt, with adjacent subarachnoid haemorrhage and physical damage to brain tissue. Diffuse damage was also seen throughout the brain, manifested as traumatic axon injury, brain swelling and haemorrhage (Gibson et al., 2009d). Non-penetrative captive bolt applied in calves (134 to 204 kg liveweight) within 5 seconds after ventral neck incision, resulted in immediately altered cortical function in all but 2 of 7 calves. These 2 calves had a period of unilaterally active EEG, lasting for 4 and 6 seconds after the stun. The other hemisphere had an EEG incompatible with consciousness, and it was suggested that incorrect positioning of the stunner might have been responsible (Gibson et al., 2009c).

In two recent studies in Germany non-penetrative captive bolt stunning was tested under routine conditions with cattle up to 500 kg slaughter weight. Hoffmann (2003) used either cartridge activated or pneumatic concussion stunning devices (Cash and EFA) and found that 12 percent out of 1248 cattle had to be re-stunned, the rate of re-stunning increasing considerably if the shooting position deviated from the midline.

Endres (2005) examined stunning effectiveness in more than 5500 mostly flecked cattle, using two different pneumatic non-penetrative devices (Jarvis and EFA). Criteria for re-stunning were regular breathing and directed focused vision. In total only 83.3 percent of the cattle, were stunned by the first blow. The rate of successful initial stuns for the Jarvis device was slightly higher. Highest re-stun rates of 20 percent were found in young bulls. Sixty percent of the 548 heads examined showed profound injuries of the frontal bone in the impact area of the bolt including inner and outer bone laminae and partly the dura mater. In all of 80 brains examined haemorrhages of varying extent beneath the impact site and around the brain stem were detected, supporting similar findings of Blackmore (1979), Lambooij et al. (1981), Finnie (1995) and Hoffmann (2003).

It was concluded that it was not possible to find the optimal relation between dimension of the concussive head of the stunner and dose of the force of the blow, to achieve sufficient effectiveness without fracturing the skull. This was because especially in the young bulls the shape and hairiness of the heads showed huge variations. Fracturing of the skull resulted in less effective stunning (Endres, 2005).

Effective re-stunning could only be achieved by penetrating captive bolt, as using a second non-penetrative blow because of swelling and fracturing did not transfer enough energy to the brain (Endres, 2005).

In cattle, sticking should be performed within 12 seconds of non-penetrative captive bolt stunning (EFSA, 2004, page 64), and if possible even earlier (Mintzlaff and Lay, 2004). Heart activity continued after slaughter as with penetrative captive bolt stunning in adult cattle and veal calves (Gibson et al., 2009d; Hoffmann, 2003; Lambooij et al., 1981).

In calves, outward signs of effective non-penetrative captive bolt stunning were described as the appearance of 5 to 15 seconds of tonic convulsions and spasms prior to relaxation, or as extensor rigidity and some generalised muscular tremors, followed by slow hind leg movements. Absence of rhythmic breathing lasted for up to 35 seconds, and absence of righting behaviour lasted for a minimum of 60 seconds (Lambooij et al., 1981; Blackmore, 1979). For adult cattle Finnie (1995) reported brief tetanic spasms followed by slow uncoordinated hindlimb movements of increasing frequency. The reduced movement in most post stun cattle compared to penetrating captive bolt stunning was described as an advantage with regard to sticking accuracy and worker safety by Hoffmann (2003) and Endres (2005).

Insufficient stunning effectiveness has also been reported in <u>sheep</u>. Blackmore (1979) could effectively stun 84 to 95 percent of lambs (3 to 4 month old) in the occipital position. The animals collapsed immediately with some generalized muscle tremors, followed by slow hind leg movements, developing into vigorous hind leg kicking. Rhythmic breathing was absent. Brain haemorrhages were frequent. Adult sheep could not be successfully stunned by this method (Blackmore, 1983), as breathing restarted between 7 and 43 seconds after the blow (Schütt-Abraham et al., 1983b).

In young Merino lambs (4 to 5 weeks old) non-penetrative captive bolt stunning has been reported to result in skull fracture in 5 out of 10 lambs. Structural brain damage, a mixture of focal and diffuse injury, was similar as in penetrating captive bolt stunning and of sufficient severity to suggest that both types of bolt are acceptable when stunning lambs (Finnie, 2000). Nevertheless bleeding should be performed as soon as possible after stunning (EFSA, 2004, page 76). For goats no data are available.

When using a non-penetrative captive bolt in sheep as well as cattle, unconsciousness should be induced with a single blow at the frontal position of the head. Subsequent shots may not be effective due to the swelling of the skin occurring from the first shot, and therefore, should not be allowed. If the first shot is unsuccessful, the animal should be stunned immediately using a penetrating captive bolt or electric current (EFSA, 2004, page 48).

Non-penetrative captive bolt devices are used to stun and kill chicken and turkeys. They are fitted with a plastic or metal concussive head. The bolt head is fired with high velocity onto the head of the chicken or turkey and causes severe structural damage to the skull and brain. The device is used in small scale slaughter plants, where prompt sticking is recommended and for casualty / emergency killing (HSA, 2005; HSA, 2004; Hewitt, 2000). Gregory and Wotton (1990b) showed that stunning chicken with a non-penetrative captive bolt resulted in pronounced changes in their visual evoked responses. A blow to the head administered by hand with a blunt instrument, e.g. a heavy pipe or stick, leads to instantaneous unconsciousness in chicken and poultry up to 5 kilograms due to cerebral commotion,

provided the blow is delivered with sufficient impact and right on the target. As efficiency depends largely on operators skill and can hardly be standardized this method is only suitable for small scale slaughter (Schütt-Abraham, 1995).

To conclude, non-penetrative concussive stunning in cattle and sheep is not satisfactory so far from the animal welfare point of view, due to the relative high failure rate. Improvements seem to be possible by developing the shape of the bolt, better fixation of the head, and standardisation of cartridge power as well as shape of the bolt in relation to different age groups and genetic lines (Moje, 2003). In Australia non-penetrative concussive stunning must be performed only on cattle, that are capable of being stunned by this method, which effectively rules out large bulls and buffaloes and also sheep, because the bony ridge and the wool on the sheep's head dissipate the force of the blow (Andriessen, 2006, cited by Adams and Sheridan 2008). Rapid sticking and if necessary back-up stunning by penetrating captive bolt are mandatory.

4.2.4 Gas stunning (poultry)

The main advantage of gas stunning is, that poultry can be stunned in groups and that handling is reduced and shackling of conscious poultry and any associated negative effects on birds welfare are avoided (EFSA, 2004, page 139).

Gas stunning is performed in some countries for Halal slaughter of chicken and turkey (Lankhaar and Nieuwelaar, 2005). Mainly a Multiphase CAS system is used, based on a biphasic principle; an anaesthetic mixture of carbon dioxide, oxygen and nitrogen (40% CO_2/30%O_2/30%N_2) is applied first for one minute in order to gently achieve a state of unconsciousness, followed by a completing mixture, with a higher level of carbon dioxide (80%) for 2 minutes, to ensure a stunning effect that lasts until death is induced by bleeding. The addition of 30 percent oxygen to the carbon dioxide in nitrogen mix was associated with increased time spent feeding and reduced headshaking in a trial to evaluate aversive reactions to different gas mixtures (McKeegan et al., 2007). For this system the presence of a heartbeat after stunning has been demonstrated though full recovery of all birds cannot be achieved (Coenen et al., 2000). If the chicken were not taken out of the completing phase after 2 minutes but stayed in the gas, heart rate could no longer be measured after 500 seconds from entering the first phase, i.e. 440 seconds after the end of the first phase and 320 seconds after exposure time would have ended in routine slaughter conditions. The chicken were considered to be dead when heart rate was 180 heart beats per minute or less and the EEG was isoelectric. This happened at 249 seconds during continued exposure in the completing phase. At this time if slaughtered under routine conditions the chicken would have left the gas atmosphere for more than one minute and neck cutting would have been already performed (Coenen et al., 2003).

A regular heartbeat was present during continuous exposure to anoxic gas mixtures (Argon/ N2) on average until 165 seconds and after mixed hypercapnic/ anoxic mixtures (carbon dioxide-nitrogen) on average until 175 seconds, but they were always reduced (McKeegan et al., 2007). Raj and Gregory (1991) showed that physiological effects of stunning gases applied for 120 seconds were not sufficient to impede the bleeding efficiency of broilers.

An evaluation of welfare of poultry stunned using the biphasic gas stunning system under practical conditions produced the following positive features:
- good welfare during bird supply to the system to warrant a gentle induction of the stunning process;
- scientifically based induction conditions and corresponding clinical appearance, which could be verified under practical conditions;

- sufficient depth of stunning assuring that in combination with given stun-stick interval and quality of neck-cutting no animals regained consciousness before dying and
- suitable process control and monitoring (Wenzlawowicz and Holleben, 2005).

Since the induction of unconsciousness with gas mixtures is a gradual process, the gas mixture should be non-aversive and the induction of unconsciousness should not be distressing to the birds (EFSA, 2004, page 140). For the mentioned multiphase CAS system concerns were raised about exposure of birds to aversive gaseous environments during the induction of unconsciousness (Raj et al., 1998) due to prolonged induction periods and potentially aversive signs (deep breathing with open beak and head shaking). Nevertheless for the carbon dioxide concentrations used, McKeegan et al. (2006) observed in a small number of birds withdrawal from the food dish, but the birds returned after 20 seconds of air reinstatement. The meaning of head shaking with regard to animal welfare is also under discussion (Webster and Fletcher, 2001). It seems likely that the response, which is rated as mildly aversive by Barton Gade et al. (2001), is primarily related to novel or alerting stimuli (McKeegan et al., 2007; Dunnington and Siegel, 1986). Scientific evidence suggests that poultry seem to tolerate concentrations up to 40 percent carbon dioxide and only concentrations higher than 40 or 55 percent seem to cause pain or a higher level of unpleasantness (EFSA, 2004, page 148).

Gas stunning is effective if there is no righting, wing flapping, vocalization or rhythmic breathing during bleeding. This is achieved by good access to the gas by every bird (no overloading), using correct dwell times and gas concentrations as well as timely cutting of both carotid arteries.

Investigations of the biphasic system under practical conditions have revealed an excellent stunning effectiveness. After neck cutting 0.003% were classified as awake, which means one bird out of 36,072 (Wenzlawowicz and Holleben, 2005).

In conclusion, gas stunning as applied by the biphasic CAS system is an effective method for stunning poultry, profiting especially from reduced handling and manipulation of live birds. Some concerns still apply such as unpleasant respiratory sensations which cannot be totally excluded, but the advantages of improved live bird handling more than counterbalance this risk.

In summary for all stunning methods the welfare benefit achieved depends on equipment and performance. Reversible stunning methods alone do not lead to the death of the animal but timely and effective bleeding must be initiated and progressed far enough to prevent temporary return of consciousness. Stunning methods in principle are pain free if applied correctly. In electrical and mechanical stunning this is warranted as shock waves or current fields travel more quickly or disrupt brain activity faster than the rate of transmission by trans-synaptic nerve pathways carrying pain signals. For gas stunning it can be achieved if a state of unconsciousness is induced first, before poultry are exposed to potentially aversive gas concentrations.

4.3   Post neck cut stunning

Stunning post neck cutting, also called "post-cut stunning" means applying a stunning method after the neck cut. If performed immediately after the neck cut, "post-cut stunning" has been considered an improvement with regard to animal welfare, compared to slaughter without stunning, as it shortens the time interval during which pain and suffering after slaughter without stunning can be experienced (Binder, 2010; Luy, 2010; Caspar and Koepernik, 2010; Gsandtner, 2005). Nevertheless even if stunning immediately follows the cut, post-cut stunning still entails considerable pain and suffering for the animal in contrast to pre-cut

stunning (Luy, 2010). This is because the painful impact of the cut is present (see also chapter 4.1.1) and post cut stunning does not cover the time immediately (within 5 s) after the cut, which will be relevant as long as the animal is conscious.

So far post cut stunning is performed using penetrating and non-penetrative captive bolt and is only described for cattle (Binder, 2010; Berg, 2007).

Gibson et al. (2009c) assessed the extent to which applying a non-penetrative captive bolt stun 5 seconds after the ventral-neck incision ameliorated the noxious sensory input caused by the incision, and showed that the stun prevented the subsequent development of responses in the EEG to noxious sensory input in most of the animals (see also chapter 4.2.3).

According to additional data from New Zealand an electrical stun following a throat cut will enhance animal welfare by accelerating the process of brain failure. Daly et al. (2010) showed that subsequent to an electrical stun applied within 3 seconds of a throat cut in sheep, spontaneous breathing, corneal reflexes and visually evoked responses were immediately abolished and did not recover before death. Although EEG recordings did not show epileptiform activity and convulsive activities did not occur in the sheep, the results were taken as evidence that an electrical stun produces immediate unconsciousness when applied promptly after the neck cut.

As described in chapter 3.3 a major concern about post cut stunning is that stunning is not carried out immediately after the first cut has been performed, but after at least about 5 seconds and often 12 and more seconds, which is considered unacceptable from an animal welfare point of view (Binder, 2010; Berg, 2007, see also chapter 3.3).

To conclude, "post-cut stunning" will avoid potential pain and suffering from the moment it is applied. This will markedly improve animal welfare in relation to slaughter without stunning during the time between the cut and loss of consciousness (see chapter 4.1.2). Nevertheless the time of the cut itself and the first seconds after the cut are to be considered a period during which the experience of pain is likely, and which is not affected by the post-cut stunning process.

5   Conclusions

After elaboration of the above considerations, the following concerns about different slaughter methods can be expressed:

5.1   Conclusions with regard to *neck cutting without stunning*

In this report no comparison have been made between the two methods of neck cutting without stunning (Halal and Shechita slaughter). Nevertheless from the available research and also experience of the involved veterinarians the same general principles apply and in routine practice improper equipment and poor performance have been found in both practices. A quantitative analysis of risks during practical performance was beyond the scope of this project.

<u>Restraining for slaughter without stunning</u>

Methods of restraining depend on animal species and size. For slaughter without stunning cattle and sheep are restrained upright, on their back or turned on their sides. In some plants cattle are restrained half-shackled, for sheep shackling is also used. Poultry are restrained by hand either on their back or laid on a table in lateral recumbency or in a shackle or cone.

- Restraining for slaughter without stunning has to ensure that the neck can be presented and stretched in a way to enable optimum performance of the cut and efficient bleeding.

- Post cut restraint is also important with regard to animal welfare:
  - the neck wound and the vessels have to stay open in the best way achievable to enable fast bleeding and prompt loss of consciousness;
  - mechanical influences on the wound (e.g. tearing forces or contact with other materials) have to be avoided when the animals have not yet lost consciousness;
  - chemical impacts on the wound and cut tissues resulting from blood or stomach content have to be minimized when the animals have not yet lost consciousness.

- In regard to all methods of restraint, suboptimal performance as well as construction and design deficiencies contribute to poor animal welfare. Slaughter without stunning requires special procedures such as stretching of the neck and post cut management that influence the choice and operation of equipment.

- Restraining heavy animals such as adult cattle represents a special challenge and can make choosing between upright restraint and turning animals on their back or side difficult:
  - Improvements are possible in all three systems with better training but also improved construction (e.g. size and pressure of neck frame, headholder, backpusher, belly plate, turning direction, turning time, turning angle, post cut management);
  - Upright restraint seems to be less stressful if the time between the moment the animal enters the device and the cut is short and if least reaction of the animals during head restraint occurs. However systematic investigations of different types of restraining devices were not evaluated within this project;
  - Though it is presumed that performing the cut in cattle during upright restraint requires more skill than in the inverted position, the data gathered during this project on the number of cuts provides no clear evidence which supports this assumption;
  - The post cut management of cattle during upright restraint is more complicated than for inverted or laterally restrained animals, because it is difficult to support the animal after the cut so that the wound stays open and the wound edges don't touch the restraining equipment;

- Blood from the cut vessels spreading over the wound and into the trachea and larynx will cause pain or discomfort with all kinds of head restraint. Stomach content spreading over the wound can be prevented in upright restraint up to the time the animals are released from the pen;
- Turning positions between upright and lateral recumbency, e.g. to 45° degrees has the potential to limit stress during turning and will avoid unnatural position;
- It can be presumed that cattle lying on their backs suffer from the weight and size of the rumen pressing upon the diaphragm and thoracic organs.

The throat cut

- It can be stated with high probability that animals feel pain during and after the throat cut without prior stunning. This applies even to a good cut performed by a skilled operator, because substantial tissue damage is inflicted to areas well supplied with nociceptors and subsequent perception of pain is not exclusively related to the quality of the cut.

- Risk factors for pain during the cut include: increased number of changes of direction of the cut, increased number of cuts, performance of back up cuts, increased cutting times, blunt blades, nicks on blades, increased diameter of the neck, increased flexibility due to insufficient tension of the neck tissue during the cut, thick wool/coat or excited animals moving their head/neck during the cut.

Time to loss of consciousness and possible impacts during this period

- Unconsciousness produced by interrupting the blood supply to the brain can be rapid, but never instantaneous. The time lag between reduced flow of blood to the brain and unconsciousness certainly depends on whether compensatory mechanisms of the body are successful or fast enough and how quickly they are eventually overwhelmed by blood volume loss. Results are quite variable and there seems to be differences between the results of scientific studies that have been conducted on a relatively small number of animals under controlled conditions and field conditions. Methods applied in slaughter houses could show even more variable results due to inconsistencies in operations and the range of individual characteristics of the animals.

- Most cattle seem to lose consciousness between 5 and 90 seconds after the cut, but even under laboratory conditions possible resurgence of consciousness have been stated for more than 5 minutes. Most sheep and goats seem to loose consciousness within 2 to 20 seconds after ventral neck cut. However investigations under practical but optimum conditions have revealed that sheep have been able to regain consciousness for up to 2 minutes. Most chickens lose consciousness between 12 and 15 seconds, but consciousness is possible for up to 26 seconds after the cut (poultry data only exists for chicken).

- All the factors prolonging the time to loss of consciousness are not fully understood and even optimum restraint and cutting cannot guarantee prompt loss of consciousness.

- Risk factors for prolonged consciousness are: bad quality of the cut (size, position, cutting technique), faulty restraint inhibiting bleeding efficiency, occlusion of vessels (false aneurysms, platelet accumulation, vasoconstriction, retraction of the cut vessels into the surrounding tissue) at cephalic and cardiac sides of the cut, suitable blood pressure gradient together with patency of alternative blood pathways to the brain or high individual regulatory capacity of the animal. It is possible that large animals such as adult cattle are more prone to prolonged consciousness than small animals. It is also

possible that the state of arousal of the animal represents a risk factor for prolonged consciousness.

- One of the main concerns about slaughter without stunning is that animals are further processed and exposed to potentially painful manipulations (e.g. subjected to follow up cuts, released from restraint or shackled) during the period they are still conscious. This can especially happen if slaughter line speed is too high to wait until the animal is irreversibly unconscious. Painful chemical impacts on the wound and severed organs such as the trachea by blood or stomach contents are also possible during this period.

Evaluation of clinical picture during the post cut period

The overall clinical picture after the cut is dominated by reactions to previous handling and manipulations (e.g. cut or restraint), symptoms of hypoxia and fading consciousness, or by signs for remaining or regaining consciousness. All three categories may be confounded by each other.

- So far patterns of clinical signs after the cut have been characterised but it is still difficult to define the exact moment the animal becomes unconscious.

- Clear signs of consciousness after the cut are "attempts to rise or to regain normal body posture", "coordinated reactions to manipulation of the wound edges" or "the animals' eyes focussing on stimuli from the surrounding and following them, which is often accompanied by repeated spontaneous blinking". If these signs are expressed or if rhythmic breathing does not cease at all, this will indicate failure of quick and permanent loss of consciousness after slaughter without stunning.

- The main concern about the post cut period is that animals may suffer due to failure to recognise signs of recovery of consciousness or inadequate measures taken when prolonged consciousness is detected.

5.2 Conclusions with regard to *stunning prior to neck cutting*

- The ideal restraining method for slaughter depends on the animals to be slaughtered, the method of stunning and the capabilities of the staff. When a mechanical or electrical stunning method is applied the restraining method must allow the secure positioning of stunning devices. Prompt back up stunning if necessary must be possible and processing for timely and effective bleeding. Inadequate restraint can lead to inadequate stunning by misplacement or interrupted application of the stunning device such as tongs or captive bolt equipment. It can also lead to late or ineffective bleeding if the animals are not processed sufficient quickly to the bleeding position.

- For all stunning methods the welfare benefits depend on equipment and performance. Reversible stunning methods alone do not lead to the death of the animal but timely and effective bleeding must be initiated and progressed far enough to prevent temporary return of consciousness.

- Electrical stunning is a humane method of rendering an animal instantaneously unconscious and with timely and effective bleeding unconsciousness and insensibility will last until death supervenes by bleeding.
  Nevertheless, the necessary technical requirements and skills must be met under routine conditions, and welfare can be poor in cases of noncompliance (lack of equipment or maintenance, wrong current parameters, incorrect positioning of electrodes, too short or interrupted current application, pre slaughter electric shocks,

inadequate monitoring and alarm setting, late or ineffective sticking, no back up stunning).

- Mechanical penetrating captive bolt stunning is a humane method of rendering an animal instantaneously unconscious, provided that the stunning apparatus is well maintained, placed correctly and the correct power of cartridges or air pressure is applied. Effective bleeding must follow the stun.
Welfare can be poor in the following cases: lack of equipment or maintenance, wrong cartridges or air pressure, incorrect position or angle of the bolt apparatus, inadequate monitoring, late or ineffective sticking, no back up stunning.

- Non-penetrative concussive stunning is an effective stunning method for poultry. In cattle and sheep the stunning efficiency is not satisfactory, however improvements seem to be possible (e.g. by developing the shape of the bolt, better fixation of the head, and standardisation of cartridge power as well as shape of the bolt in relation to different age groups and genetic lines). Rapid sticking and if necessary back up stunning by penetrating captive bolt are mandatory. Beyond insufficient stunning effectiveness, welfare can be poor in the following cases: lack of equipment for restraint or stunning, inappropriate maintenance, wrong cartridges or air pressure, incorrect position or angle of the bolt, inadequate monitoring, late or ineffective sticking, no back up stunning.

- Gas stunning with the biphasic CAS system is a very effective stunning method for poultry profiting especially from reduced handling and manipulation of live birds. Some concerns still apply and unpleasant respiratory sensations cannot be totally excluded, but the advantages of improved live bird handling more than counterbalance this risk. Risks of reduced stunning effectiveness increase if access to the gas is impaired (e.g. overloading), dwell times or gas concentrations are inadequate, or sticking is too late or ineffective.

- Stunning effectiveness can be 100% under routine conditions. Insufficient effectiveness can occur in single animals due to abnormal behaviour, anatomical or physiological variations such as hyperexcitability or thickness of the skull bone. This can hardly be avoided and requires competent monitoring by the responsible staff and adequate back up stunning. Lack of back up stunning is a welfare concern.

5.3 Conclusions with regard to *post neck cut stunning*

- Hazards associated with post cut stunning include the risks of improper head restraint when performing the cut or the stunning method. Welfare will be especially poor if optimal performance of the cut is not possible, if stunning cannot be performed immediately after the cut, and if the necessary equipment and skills for effective stunning are lacking.

- Post neck cut stunning will avoid potential pain and suffering from the moment it is applied. Nevertheless pain can occur at the time of the cut itself and the first seconds after the cut, and this will not be prevented by the post-cut stunning process.

5.4 Overall conclusions

The aim of the present report was to summarize, evaluate and discuss - in an unbiased and comparative fashion - animal welfare concerns from the viewpoint of veterinary sciences in relation to slaughter practices including *neck cutting without stunning*, *stunning prior to neck cutting* and *stunning post neck cutting*. Scientific findings should be taken into account as well as experience gathered by veterinarians under practical conditions.

- All slaughter methods bear the risk of inadequate equipment or lack of sufficient knowledge or skills. They should be compared either if performed under optimum conditions or including an evaluation of the specific risks under practical conditions.

- There is considerable room for development and improvement with regard to management of the implicated risks for all slaughter methods.

- There is a need to define standard operation procedures for all slaughter methods. In regard to *stunning prior to neck cutting* especially diagnosis of failed stunning is required and measures to prevent inadequate stunning efficiency must be taken. Additional indicators during *neck cutting without stunning* to determine final loss of consciousness and actions to be taken in cases of prolonged consciousness are also needed.

- For all slaughter methods excited animals represent a special risk. In excited animals exact application of the stunning devices and the cut is more difficult and this can cause additional pain and suffering. Moreover, it is possible that stunning effectiveness can be impaired and that there is an increased risk for prolonged consciousness during slaughter without stunning.

- During *neck cutting without stunning* and often during *post neck cut stunning*, restraint is complex and imposes more stress and strain on the animal than during *stunning prior to neck cutting*. More manipulation of the animal is required to achieve the right position for neck cutting, including stretching the neck in red meat species. In addition, improved post cut management is needed after *neck cutting without stunning* to achieve optimum bleeding and also avoid the risk of mechanical and chemical stimuli on the wound surfaces.

- It can be stated with high probability that unstunned animals feel pain during and after the throat cut without prior stunning.

- If a *reversible stunning method* is successfully applied the animal will lose consciousness immediately (except for gas stunning) and will not feel potential pain during the cut and subsequent bleeding. If *neck cutting without stunning* is used, unconsciousness will occur after the brain function is lost due to lack of perfusion with blood.

- There is a critical period after the incision, during which an unstunned animal may temporarily perceive pain and distress before it becomes irreversibly unconscious due severe blood loss. In addition multiple cuts could increase the potential of inflicting further pain. This period represents a special risk.

- *Reversible methods of stunning prior to neck cutting* bear the risk of regaining consciousness if sticking is performed too late or if bleeding quality is low. For *neck cutting without stunning* there is as well a risk of drifting into consciousness again if compensatory mechanisms of the body are successful and not overwhelmed by volume losses. It might be argued that both risks are comparable. However, it has to be taken into account that for *neck cutting without stunning* there is no safety margin.

Moreover, even considering the variability of routine slaughter conditions, usually no "back up"- stunning is performed after slaughter without stunning.

- *Stunning post neck cutting* will avoid potential pain and suffering from the moment it is applied. This should markedly improve animal welfare in relation to *neck cutting without stunning* during the time between the cut and loss of consciousness. Nevertheless the time needed to perform the cut and the period after the cut are not affected by post-cut stunning.

Although it was not within the scope of the project to perform a risk assessment approach a comparative analysis of the risks is shown in Table 7.

Table 7: Comparative ranking of risks with regard to compromised animal welfare due to different slaughter methods

Hazard	neck cutting without stunning	stunning prior to neck cutting	stunning post neck cutting
Pre slaughter handling stress	High	High	High
Restraint stress and injury	High	**Low**	Intermediate
Inadequate equipment	High	High	High
Lack of knowledge or skills	High	High	High
Pain and suffering during the cut	High	**Low**	High
Pain and suffering during the post cut period	High	Low	Intermediate
High slaughter line speed	High	**Low**	**Low**
Failure to diagnose of prolonged consciousness or inadequate stunning	High	Intermediate	Intermediate
Need for back up stunning in case of prolonged consciousness/ or failed stunning	High	**Low**	**Low**

Without making a value judgement it can be stated that *neck cutting without stunning* poses the highest risk for animal welfare because restraining for the cut and during bleeding imposes extra manipulation to the animal. Additionally, pain, suffering and distress during the cut and during bleeding are highly likely. The latter is partly reduced during *stunning post neck cutting*, which represents an intermediate risk for animal welfare. Although stunning methods themselves involve risks to animal welfare which have to be managed, *stunning prior to neck cutting* represents the lowest risk for overall compromise of animal welfare.

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Glossary

Bleeding: cutting the major blood vessels supplying or draining blood in the brain (see also **Sticking**).

Captive bolt stunning: Stunning by concussion of the brain through an impact of the bolt with the skull of animals.

Chest/ (pre-) thoracic sticking: severing major blood vessels emerging from the heart by inserting a knife in front of the brisket or sternum (double cut: first the skin, then, with another knife, the vessels).

Conventional slaughter: slaughter after stunning

Corneal reflex: blinking response to touching the eyeball indicating an active brain stem or light anaesthesia.

Death: a physiological state of an animal, where respiration and blood circulation have ceased as the respiratory and circulatory brain centres in the Medulla Oblongata are irreversibly inactive. Due to the permanent absence of nutrients and oxygen in the brain, consciousness is irreversibly lost. In the context of application of stunning and stun/kill methods, the main clinical signs seen are permanent absence of respiration (and also absence of gagging), absence of pulse and absence of corneal and palpebral reflex.

Electric stunning: Stunning by electric current passing through the brain. Electric stunning may be carried out as a reversible stunning method or as an irreversible stunning method (see also **Stun**, **Stunning** and **Stun/kill or stunning/killing**).

Exsanguination: see **Bleeding** or **Sticking**

Gas stunning: Stunning by exposing animals, to a predetermined gas mixture contained within a well or tunnel.

Halal slaughter: Muslim slaughter method (see **religious slaughter**). Meat declared fit for the consumption by Muslims is called Halal; unfit meat for the food of Muslims is called Haram.
Halal slaughter is slaughter of an animal that is lawful according to Islamic law (halal) and that is alive at the time of slaughter. The slaughter process must be carried out by a trained Muslim and begins by invocation of Allah (Bismillah, Allahu Ekber, In the Name of Allah).
Halal slaughter is considered complete if the trachea, oesophagus and main arteries and veins are cut in the neck region (at least three of the four structures oesophagus, trachea and both carotid arteries must be cut completely).
The instruments for slaughter must be sharp to ensure the most stress-free and quick halal slaughter possible and optimal bleeding.

Hoisting for carcass processing: lifting an unconscious animal or carcass to an overhead rail, normally using shackles and a chain attached to a leg, for the purpose of bleeding or processing.

Insensible: inability to perceive stimuli (unable to feel pain).

Jewish method of slaughter: see **Shechita**

Muslim method of slaughter: see **Halal slaughter**

Neck cutting: severing major blood vessels in the ventral neck region (skin and vessels cut simultaneously).

Religious Slaughter: means slaughter according to religious rules (see also **Halal slaughter**, **Shechita**). Religious slaughter does not necessarily mean that slaughter is carried out without stunning (see also **Stun**, **Stunning**).

Restraining: means restricting the movement of an animal/ holding the animal in a correct position, so that a procedure (e.g. sticking or stunning) can be carried out accurately.

Rhythmic breathing: regular breathing indicating an active brain stem, and can indicate the start of recovery after stunning.

Schächten (German term)**:** This colloquial German term covers both the religious slaughter according to Islamic as well as to Jewish rules. The term "Schächten" has to be understood to mean both a "religious slaughter without stunning" as well as a "religious slaughter with stunning".

Shackling: attaching a shackle to the hind leg(s) of an animal to allow it to be carried away for further procedures like stunning or bleeding.

Shechita (Schechita): Jewish slaughter method (see **religious slaughter**). Meat declared fit for consumption by Jews is called Kosher; meat unfit for consumption by Jews because it was not slaughtered properly is called Nevailah. Colloquially, all unfit meat is also called Treifah, although that term has a more precise meaning. The Jewish slaughter method, shechita, is mainly characterized by the slaughter of the animal being carried out by a highly trained, devout Jew using a perfectly smooth knife to slice the throat in a continuous motion resulting in rapid exsanguinations and loss of consciousness. For the meat to be kosher, the animal must free of specific physical defects (i.e. not a treifah) at the time of slaughter as determined by a post-mortem examination by a specially trained rabbi. Thus, shechita is but one step in the production of kosher meat, which includes the selection of a kosher species, its proper slaughter, the post-mortem inspection, and the removal of certain non-kosher sections.

Slaughter: means the process of bleeding to induce death, usually by severing major blood vessels supplying oxygenated blood to the brain.

Sticking: act of severing major blood vessels (also see **neck cutting**, **chest/(pre-)thoracic sticking**, **bleeding**).

Stun or stunning: stunning is a technical process that each animal is subjected to. Its purpose is to induce immediate unconsciousness and insensibility in animals, so that slaughter can be performed without avoidable fear, anxiety, pain, suffering and

distress.

Stunning methods can be reversible or irreversible (see also **Stun/kill or stunning/killing**). Stunning is performed before slaughter except in the case of **post-cut-stunning**, where it is performed immediately after the cut.

Stun/kill or stunning/killing: process of rendering animals unconscious first and then inducing death or achieving these simultaneously.

Unconsciousness: Unconsciousness is a state of unawareness (loss of consciousness) in which there is temporary or permanent disruption to brain function. As a consequence the individual is unable to respond to normal stimuli, including pain.

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